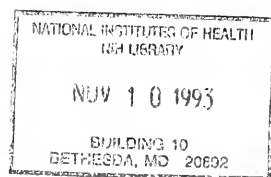


RC  
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1993

Fiscal Year 1993  
Intramural Annual Report  
National Institute on Aging  
National Institutes of Health





**Fiscal Year 1993**

**Intramural Annual Report**

**of the**

**National Institute on Aging**

**National Institutes of Health**

RC  
950  
N277  
1993



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FY 1993

INTRAMURAL ANNUAL REPORT

NATIONAL INSTITUTE ON AGING

**Intramural Research Program**

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Overview of the Epidemiology, Demography and Biometry Program	EDBP
--	------



OSD

RBB

LDB



## **Annual Report of the Office of Scientific Director National Institute on Aging**

The Scientific Director of the NIA is responsible for the overall direction and quality of research conducted by the Intramural Research Program (IRP) which includes nine laboratories and branches in Baltimore and the Laboratory of Neurosciences located in the NIH Clinical Center in Bethesda. The Office of the Scientific Director oversees the central administrative and support activities necessary for the successful operation of the Intramural Program. These activities are carried out by the Administrative, Procurement, and Information Offices, and the intramural program Personnel Office.

Fiscal Year 1993 was a productive one for the IRP. Selected highlights from the laboratories and branches follow.

### **Repair of Oxidative DNA Damage**

Scientists in the Laboratory of Molecular Genetics have developed assays to detect oxidative lesions in specific genes and to quantify their formation and repair. These assays work well in vitro and are being used increasingly in vivo to characterize the gene- and strand specific DNA repair of 8-OH guanosine and other lesions in human and hamster DNA.

In the past it was thought that there is no DNA repair in mitochondria. However, this laboratory's investigators found that these organelles do have repair capacity. They are capable of repairing DNA lesions created by monofunctional alkylating agents, but not lesions resulting from ultraviolet induced pyrimidine dimers. Further investigations are underway to determine whether the common deletions in mitochondrial DNA, seen in aging and other conditions, could be due to a localized deficiency in DNA repair.

### **Heat Shock Gene Expression**

Investigators in the Laboratory of Molecular Genetics have developed a cross-transplantation model to determine whether the aging process is inherent to the aorta or whether it is the aorta's environment that is responsible for the age-associated decline in stress-induced HSP70 expression in this tissue.

Early results indicate that when aortas of aged animals are transplanted to young animals, they show enhanced expression of HSP70 mRNA relative to native aortas of aged animals. In contrast, when young aortas are transplanted to aged animals, they show a marked attenuation in the response to restraint. Thus, it seems the environment in which the aorta resides is a major factor in determining the level of HSP70 expression in vessels of stressed animals.



## **Motor Control Decline with Aging**

Scientists in the Laboratory of Cellular and Molecular Biology find that as animals, including humans, age there is a decline in the brain in the number of dopamine receptors ( $D_2$ ) important for motor control.

Because of receptor loss, nerve cells in the corpus striatum become grossly dysfunctional in people with Parkinson's and Huntington's diseases. Similarly, during normal human aging the dopamine system deteriorates, affecting motor control abilities including balance, posture and gait. In half of the cases, the decline in receptors is due to the age-associated loss of nerve cells in the corpus striatum.

The loss of the other half is molecular—there is less expression of the gene for this receptor. This research seems to confirm earlier studies suggesting there are specific alterations in gene expression with aging leading to lower production of mRNA in older individuals.

## **Dedifferentiation of Smooth Muscle Cells with Age**

In atherosclerosis, smooth muscle cells migrate from their normal position in the vessel wall into the vessel lumen where they proliferate and obstruct blood flow. Laboratory of Cardiovascular Sciences researchers have found that with age smooth muscle cells become highly motile and invasive, producing specific proteases that destroy normal vessel structure and allow their movement. A key to this process is the dedifferentiation of the smooth muscle cells by certain negative regulators of gene expression, the helix-loop-helix ID protein which switches the cells' phenotype. Various compounds have been shown to stabilize the dedifferentiation of the smooth muscle cells and could serve as prototype "drugs" to reduce vascular disease.

## **Physical Conditioning and Heart Aging Changes**

Laboratory of Cardiovascular Sciences investigators studied 56 male participants in the BLSA and 12 highly trained athletes in an effort to test the hypothesis that age-associated reductions in physical conditioning status mediate the decrease in left ventricular diastolic performance with advancing age.

The researchers measured radionuclide ventriculographic peak filling (PF) rates at rest and throughout graded upright cycle ergometry. At rest, at 50% of maximum workload and maximal effort, PF rates declined with age but were similar in both older athletes and their more sedentary peers. This suggests that the age-associated decline in early diastolic filling observed at rest and during exercise cannot be reversed even by long-term endurance training.





## **GHRF Restores Hormone Production and Improves Strength**

A decline in growth hormone (GH) levels parallels the loss of muscle as well as the development of frailty. Previous studies have shown that the administration of recombinant growth hormone reduced body fat and increased muscle mass in older individuals with low GH levels. Recent collaborative studies by NIA Endocrinology Section scientists and Johns Hopkins investigators have shown that a natural stimulator of growth hormone production, growth hormone releasing factor (GHRF) restores the normal pattern and level of GH in older individuals. Significant increases in muscle strength were apparent after six weeks of treatment. This suggests such factors could be used to reverse some age-associated diseases and disabilities.

## **Aging Effects on Exercise**

Scientists in the Laboratory of Cellular and Molecular Biology testing 26 Baltimore Longitudinal Study of Aging volunteers who performed a constant isometric exercises, found no significant changes related to aging. Subjects performed the exercise protocol, including contraction at 30% of the individual's maximum, for three minutes. There were no systematic variations of pH or PCr/p<sub>i</sub> as a function of age. These results suggests that age per se has little effect on forearm muscle metabolism during isometric exercise at a fixed relative workload.

## **Age and Thermoregulation**

Older people risk developing cardiac dysfunctions in response to cold stress. This rise is enhanced when the subject exercises in the cold. Recent Laboratory of Behavioral Sciences research suggests that when old animals are exercised and then confronted with a cold stress they show a decrease in cold tolerance. However, this effect is eliminated in old animals by gradually introducing them to exercise.

Also, there are diurnal variations in cold tolerance in adult and old mice. Mice are nocturnal animals and have poorer cold tolerance and lower metabolic heat production in the afternoon. Exercise prior to a cold test reduces the diurnal difference in cold tolerance, but not the difference in metabolic heat production. This finding suggests that heat conservation may be poorer during circadian rest, and that exercise may "prime" the vasculature to respond more effectively to cold stress at this time.

## **Retarding Age Related Deterioration**

Recent studies have shown that part of the glucose tolerance loss occurring with age is due to secretory abnormalities of the  $\beta$ -cell of the pancreas.



Research in the Diabetes Unit, Laboratory of Clinical Physiology, shows that not as many cells release insulin with age and the amount of insulin secreted per cell is decreased. In conjunction with this, the messenger RNA for insulin decreases with age more than other islet messages. There also is a decline in REG gene expression with normal aging. This unit is continuing to tease out the trophic factors responsible for maintaining normal  $\beta$ -cell and islet function, and to look for agents that may modify this seemingly inevitable decline in function over time.

### **Reversing Bone Loss**

Laboratory of Biological Chemistry investigators have found a number of significant differences in the response of old bone to trauma, in the number of stem cells, and in the response of growth factors. Now they also have made the potentially important finding that minocycline, a tetracycline derivative which accumulates in bone, increases the bone density of old animals. At the same time, little or no effect was seen in adult animals, suggesting that an age-associated deficit was reversed.

Minocycline is used clinically to treat acne and is currently being tested in multi-center trials for its ability to inhibit the progression of rheumatoid arthritis. It is under study also as an inhibitor of bone loss in periodontal disease. In addition to possessing antibiotic activity, minocycline has been shown by researchers elsewhere to inhibit collagenase. The NIA scientists postulate that a reduction in bone resorption secondary to an inhibition of collagenase might help explain the increase of bone density in old rats.

### **Risk Factors for Age-related Ocular Change**

A study of 719 Baltimore Longitudinal Study of Aging Participants aged 40 and over showed that low levels of serum Vitamin E are a risk factor for nuclear opacities, but no other measures of antioxidant status, nor a combined antioxidant index, were associated with cataracts. Also, no evidence of increased risk from either macular degeneration or high risk macular characteristics were found associated with elastotic degeneration. Females were significantly less likely to have high risk macular characteristics compared to males. Finally, the BLSA collaborators found that low levels of serum vitamin E status were a risk factor for age-related maculopathy, as was low antioxidant status.

### **Transmissibility of Alzheimer Disease**

Laboratory of Neurosciences researchers recently investigated reports that Alzheimer disease may be transmissible. To test this possibility, blood was obtained from 21 unaffected first degree relatives (families also had another affected AD member), 10 demented patients with clinically diagnosed AD, and 21 healthy controls without a familial history of neurologic disease.



Hamsters were inoculated intracerebrally with these blood products. No hamster in either the first or second passage developed neurological signs consistent with spongiform encephalopathy which had been reported transmitted to hamsters through blood inoculations done in other laboratories. This work suggests that Alzheimer disease is not due to a transmissible agent.

### **Visuospatial Attention in DAT**

Reaction time measurements in patients with dementia of the Alzheimer type and in controls during a letter discrimination task were made in the Laboratory of Neurosciences this year. Subjects were given both valid and invalid clues. There were no differences between controls and early DAT patients with valid clues. However, invalid cues caused greater slowing of reaction time in the patients. Performance was correlated with asymmetry of metabolism in the superior parietal lobe in DAT patients, but not in controls. The results show that focusing of attention to spatial location is intact in early DAT, but disengagement of attention is impaired. The researchers think this attentional dysfunction may be linked to disruption of cortical networks linking the parietal and frontal lobes.

### **Inactivity Risk for Morbidity and Mortality**

Data from the Established Populations for Epidemiologic Studies of the Elderly (EPESE) were used by Epidemiology, Demography and Biometry Program staff to look at the association between recreational physical activity among physically capable older people and functional status, incidence of selected diseases, and mortality over three and six years.

This study showed a high level of recreational activity reduced the likelihood of mortality over the period surveyed. A consistent relationship between activity and new myocardial infarction, stroke, incidence of diabetes, or angina was not found after three or six years. These findings suggest that physical activity offers benefits to physically capable older adults, primarily in reducing the risk of functional decline and mortality.

### **Information Services**

Staff prepared several highlights for use in reports to Congress, for NIA Research Highlights, NIH News and Features, or to be sent to the media and interested citizens. An article in the former publication resulted in an interview by the *Chicago Tribune* on the bone research of Dr. Liang. Other publicity generated for the 1993 Shock Lecture resulted in The Discovery Channel (national cable) being provided with a videotape of the lecture taped by this office. Three NIA Director's Reports to NACA were prepared as was the annual international activities report. Staff researched and published three issues of the BLSA newsletter for participants, and 12 *Geron News* for NIA staff.



IO staff assisted in arranging for videotaping, interviews, and editorial input for recruitment activities involved with estrogen and growth hormone research and the new perimenopausal and cardiovascular initiatives. Alumni background was provided for an intramural research report requested by Dr. Liotta. Responded to *Glamour* magazine with slides used for the Woman of the Year Award presented to Dr. Healy. Staff began new columns in *Geron News* on security and safety issues in response to a service survey and Human Relations Committee interests. Coordinated Annual Report and NIH Directory and Bibliography submissions and publication.

The Information staff responded to 135 public inquiries and 115 media contacts as of August. The latter included requests from international, national and regional media. International contacts were made with Austrian Broadcasting Corp., Netherlands Broadcasting, Science and Technology (Netherlands), Public TV of Spain, Brazilian TV, Voice of America, Canadian TV and radio. National contacts included *The New York Times*, *Washington Post*, *U.S. News and World Report*, *USA Today*, *Parade*, *People*, CBS-TV, PBS-TV, ABC 20/20, *American Health*, *Self*, *JAMA*, *Working Women*, *AMTRAK Magazine*. Regional requests came from WJZ-TV (filmed GRC material for 3-part series), WRC and WUSA (Washington), WBZ Radio (Boston), WABC-TV (NYC), WBAL-TV (Baltimore), Miami Channel 4 (NBC), KARE-TV (Minneapolis), and KOA Radio (Denver). Staff helped arrange and execute briefings/tours for 180 people both at the GRC and off campus, including Chinese, Japanese, Dutch, and Georgian Republic visitors; and, Gerontological Society of America meeting attendees.





ICS

LOMB

RRB

LSB

LBS

LBC



**Annual Report Of The Research Resources Branch  
National Institute on Aging**

**Technical Development Section**

The TDS has experienced phenomenal growth in the number of users, as well as the number of systems, becoming part of the building network. This increase was undoubtedly caused by the connection to the world-wide network called InterNet. This was the long awaited 56Kb telephone link to the universally used network. This facility allows users to access resources, such as E-Mail, Telnet and FTP, on a world-wide basis. In addition, there is access to services that are both time saving and cost effective. For example, the use of Grateful Med, a literature and search document retrieval service, which is available to PC owners who are correctly configured. We have been rapidly configuring systems to the users' needs. As an example, PCs can boot in a DecNet or TCP/IP environment depending on the need for network resources. We have connected the Administrative, Personnel and EEO offices to the ethernet, via TCP/IP protocols. This enables them to have direct access to the LanManager software in use by NIA offices, and to the appropriate data bases, such as Status of Funds within the NIA Bethesda system. We have exchanged E-Mail addresses with the NIA Bethesda personnel, allowing users to easily communicate and exchange information. Still needed is the resolution of problems with areas on campus, communicating directly with the LanManager software.

Over the past year, "travel software" was installed on 20 PCs for access to the Bethesda travel order system. It is now possible for each area to process their own travel orders locally.

Two RF-73 disk drives were added on the central VAX cluster which allowed us to increase the space allowed for scratch areas, as well as the ability to create a file service for the storage of large amounts of data from the Laboratory of Molecular Genetics. The latter enhancement for the LMG network has proven extremely reliable and a convenient way for researchers to access their data. the LMG system developed last year has increased to 27 users and PCs.

In the area of telecommunications, the TDS is involved in negotiations with the TCB concerning the replacement of the GRC telephone system. This was necessary for two reasons: 1) the need for voice mail, and 2) the fact that the Bethesda phone system was replaced by a new switch which eliminated the direct dial ability from GRC to Bethesda. It is unclear at this time whether we will proceed with a complete upgrade or increase the number of Merlines to one per branch/laboratory, and add voice mail to those units that require its use.

This year, installation of video conferencing capability was completed at the GRC. This allows staff to originate or receive conferences with other sites at NIH as well as those across the



country. All areas currently broadcasting from NIH can be received by the GRC, including Masur Auditorium. We can also hold executive staff meetings between Building 31 and our conference rooms, reducing the need for GRC staff to constantly travel to Bethesda for these frequent meetings. So far, his system has been well received and it would appear it will receive heavy utilization.

A small PC-based system that controls the temperature and  $pO_2$  in a cell culture incubator, was developed for the Laboratory of Cellular and Molecular Biology. Motion detectors were modified and implemented for monitoring primate activity in the Poolesville Diet Restriction Study of the Molecular Physiology and Genetics Section, LCMB.

A continuing effort was devoted to the expansion of a PC-based system to collect calcium and electrophysiological data for the Laboratory of Cardiovascular Sciences. The latest development is a digital integrator that will accurately measure the 4 microsecond-wide fluorescence pulse during calcium measurements.

### **Photography and Arts Unit**

In addition to the normal production of negatives, slides, prints, and poster materials, the computer graphics system was expanded to include the development of new software for use in the Unit. This new software enables the Unit to produce graphs which closely match any type of variation presented to the Unit. Plans are being made to make this software available to any VAX user with the proper hardware. In addition, new hardware has been ordered for the VAX machine room to be used by anyone in the building.

An increased demand for service this past year was matched by increased capacity of the computer graphics system. Considerable time was saved, not only because some of the work was done by those outside the Unit, but also because of the time expended on the computer is far less than the time needed for conventional processes. The MacIntosh continues to help the Unit add to its computer graphics capability. In addition, a color thermal dye transfer printer was added this year to increase the quality of work being performed by this department.

### **Library Unit**

This year we focused on improving our bibliographic search interlibrary loan service. Special efforts were made to facilitate the GRC staff, with direct online access to search Medline databases and to order documents electronically from the NIH Library. To date, more than 30 GRC employees have registered as users of this NIH-wide service. Access can be gained through the VAX system. We also have offered training sessions to familiarize staff with the Medline databases and searching techniques, including traditional Medline mod and its simplified PC-version program, Grateful Med.



Regarding the interlibrary loan service, intramural scientists may choose between two methods to order books and articles. Users may choose to use the OnLine Public Access to Catalog (OPAC) system or the Lonesome Doc Module on Grateful Med. Either way, the NIH Library serves as the primary provider for requests.

As an intermediary provider, the GRC Library has signed up as a DOCLINE user with the National Library of Medicine. Interlibrary loan requests can be produced by library staff, or by intramural staff via personal computers. Through DOCLINE, all interlibrary loan requests can be routed electronically from the GRC Library to various other libraries, one after another, until each request is filled. This year, we joined the Maryland Interlibrary Loan Network-MILNET. MILNET broadens the scope of perspective suppliers for our loan requests by including academic and public libraries.

Despite the availability of the personal ordering system, the GRC Library made 1,200 interlibrary loan requests over the last year. The real workload exceeds this amount since this figure does not include the increasing number of materials we have handled for GRC staff who generated requests from their own PCs. Similarly, the GRC staff registered 1,353 registered requests on the Library's Standalone-the compact disc version of Medline. Such heavy usage justifies its continued existence in the Library.

This summer we began the process of shifting our journals. When this is completed, journals published before 1986 will be stored in the basement area. Our journal holdings were updated in June and we are conducting a survey soliciting a list of the most needed journal titles used by each GRC laboratory, branch and section for the Library to keep or to add to its collection. This survey was initiated as an attempt to maximize usage of our journal collection and to minimize dependency on other libraries for photocopies of articles. The completed survey results will help determine which journal subscriptions will be kept or dropped in the future.

Statistics for other routine operations include: 200 books ordered and catalogue; 258 journal volumes bound; and, some 150 Library users from other institutions during a three month survey period.

### **Animal Resources Section**

This past year, the Animal Resources Section devoted many hours to continuing education. The majority of ARS staff are trained and prepared to substitute in several areas, so that under any circumstances reliable help is available. The following investigator workshops were held: 1) Using Animals in Intramural Research; 2) Animal Handling and Restraint; 3) Rodent Anesthesiology; 4) Microchips-Permanent Rodent Identification; and, 5) Protective Clothing and Primate Training for the Investigator. Numerous other demonstrations were provided on an individual basis, involving approved animal study proposals requiring technical assistance.





A new computer program was designed, to be implemented soon, to facilitate the disposition of all animals maintained at the GRC. All animal procurements, production colony and rodent colony issuances, are monitored and automatically assigned to the appropriate protocol number via computerized data entry. This program will enable the ARS to effectively track all experimental animals to assure compliance with all Federal regulations.

The ARS continues to collaborate with the GRC staff, resulting in ARS staff co-authoring four papers submitted for publication. The information concerning the Pathology Characterization of GRC Wistar Rats has been assembled and accepted for publication. Two papers on our work with mini-pigs were also published, and one paper, on our Aging C57BL/6J Mouse Colony, has been accepted.

The ARS maintained full accreditation for the 16th year with the American Association of Accreditation for Laboratory Animal Care. A site visit is expected early next year. In an effort to keep up-to-date on all the most current regulations concerning our animal care and use program, the ARS represents the NIA at the NIH Animal Research Advisory Committee and Animal Program Advisory Committee meetings. Creating a closer working relationship between the NIH and the GRC has proven of great value especially with regard to new and ever changing Federal regulations that mandate the way our animal care and use program operates. The ARS also collaborates with the National Institute of Drug Abuse Animal Care and Use Committee and Animal Services Division by supplying expertise and technical assistance to their program.

New computerized dispensing equipment has been added to all ARS racks and tunnel and bottle washers to dispense detergents and acids during the sanitation process. The multiple programs enable ARS personnel to accurately control chemical use and retrieve recorded data for the total number chemical product used. Through this program we are able to assure compliance with USDA sanitization regulations meet local ordinances for neutralization of chemicals prior to discharge to Maryland waste water treatment systems.

The following details population inventories and the daily ARS census. This year, 5,516 mice and rats were issued from the aging rodent colonies. Care was provided for an average daily population of 13,472 rats, 10,218 mice, 6 hamsters, 8 rabbits, 19 dogs, 5 guinea pigs, 9 monkeys, and 2 pigs. In addition to these stock animals, approximately 13,156 rats, 6,934 mice, 24 guinea pigs, 21 rabbits, 16 hamsters, 6 pigs, 4 monkeys, and 10 dogs were received, housed and cared for in the section.

Three ARS staff achieved outstanding EPMS ratings and 10 employees received excellent EPMS ratings for 1992.



## **Instrument Design and Fabrication Section**

Over the past year, along with many 30 minute to two hour construction and repair jobs, The IDFS staff designed, fabricated and installed equipment for various units of the GRC. The following are representative of the different jobs performed: 1) A room was set up with a wall mounted swivel and harness to monitor dogs and swine; 2) A Magnetic Resonance Imager was upgraded with all restraining devices needing to be redesigned and fabricated, In addition, section employees spent considerable time preparing space for a new MRI. Many pieces of laboratory equipment were repaired and recalibrated.

Due to the reorganization of various GRC laboratories, the IDFS staff has continued to dismantle old laboratories to set up new ones. Every time this is necessary, the IDFS has to relocate wiring for computer terminals and move telephones. This process is quite complicated and time consuming. Various other projects were successfully completed during the year.



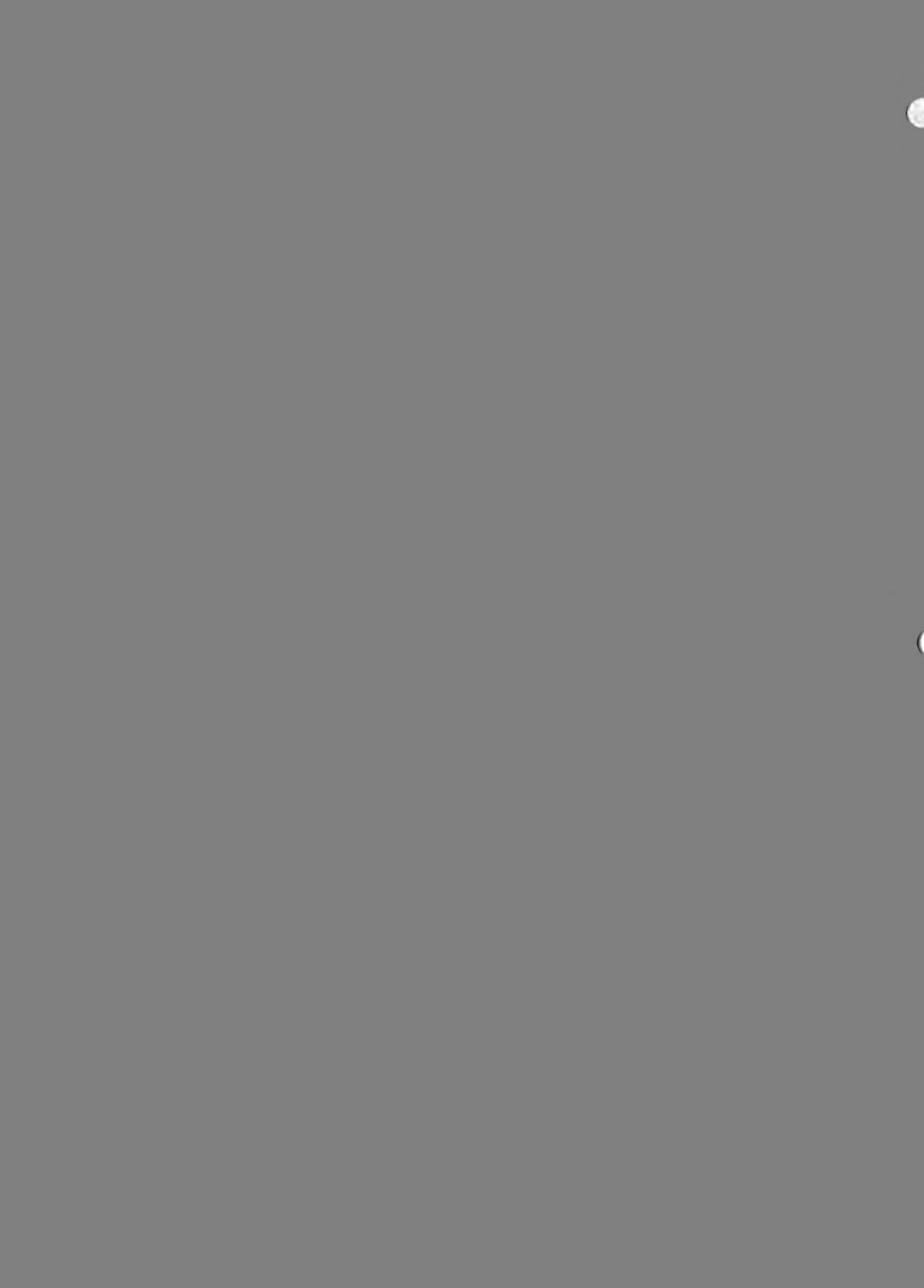
LCS

LCMB

LCE  
LSB

LBS

LBC



## ANNUAL REPORT OF THE LONGITUDINAL STUDIES BRANCH

### NATIONAL INSTITUTE ON AGING

The most far reaching FY 1993 activities in the Baltimore Longitudinal Study of Aging (BLSA) were related to the continuing development of new research initiatives related to aging in women and minorities. The Vascular Initiative consists of a series of interrelated studies of racial differences in cardiovascular function and cerebral blood flow in African-American men and women and their Caucasian counterparts. Two of the studies, developed by scientists in LBS, LCS, LSB and LCP, were initiated in FY 1992 and three others, named in this Annual Report, were initiated in FY 1993. The Perimenopausal Study initiated in two sections of LCP (APS and ERS) is designed to measure changes in hormone levels in African-American and White women as they go through menopause and to follow the consequences of the changes, such as losses in bone density and strength, changes in body composition, and development of cardiovascular and metabolic disease. In addition to the new studies, many investigators have developed hypotheses relative to age associated racial differences in existing BLSA studies, e.g., strength, prostate growth, visual function, activity patterns, nutrition, and self reported activity levels.

Funding and support for the research initiatives on women and minorities were provided by special resources established by the Scientific Director, NIA, on the basis of recommendation by the BLSA Steering Committee. In addition to the projects associated with research on minorities and women, the BLSA Steering Committee approved a revised protocol on oral physiology and aging which extends the research to all BLSA participants. The newly approved projects are listed later in this report.

The role of the LSB in the BLSA is to operate and manage the Study and to perform research with it, using both historical and currently collected data. Highlights of these operations and research activities follow.

### BLSA OPERATIONS

#### POPULATION DYNAMICS

The number of active BLSA participants is larger than ever; 508 women and 665 men.

The second systematic followup of inactive participants was completed on December 31, 1992; the data have been used by several investigators; and several manuscripts are in preparation.

Vigorous recruitment efforts for participants in the Perimenopausal Study and Vascular Initiative are underway including the final steps required to implement a contract for recruitment of the needed minority and female participants.

#### DATA MANAGEMENT

The BLSA Common Data Set was developed and implemented.

The data base and forms for the Interval Medical History were developed and put in place.

An on-line system for administering the Physical Functioning Inventory is now in use with active participants.

#### CLINICAL HEALTH EVALUATION

An improved system of demographic information for BLSA participants was implemented and made available.





The Interval Medical History and appropriate branching queries were implemented.

The Physical Functioning Inventory was implemented in the active participants.

#### LSB RESEARCH WITH THE BLSA

##### SENSATION AND PERCEPTION

Vision--Age changes in acuity and stereopsis in women similar to that of men.

Vision--First report published showing that low levels of serum Vitamin E is associated with greater risk for nuclear opacity.

Vision--New study initiated on race and gender differences in blood pressure/intraocular pressure relationships in relation to visual field sensitivity.

Hearing--Rate of age associated hearing loss in women is approximately half of that in men. However, the pattern of loss is similar except for a reversal in the amount of loss in the lower frequencies.

Hearing--An instrument is being developed to study sex differences in noise exposure in occupational and nonoccupational activities.

##### HEALTH/DISEASE RELATIONSHIPS IN AGING

Strength--New protocol implemented.

Self-Reported Activity--Level and range of self reported activities are less in persons of all ages who have preexisting medical problems.

Pulmonary Function--Accelerated rate of pulmonary decline, in addition to initial lower levels, are associated with greater probability of cardiovascular death in men and in greater cardiovascular morbidity in men and women after other known risk factors are accounted for.

Prostate--New prospective study of growth implemented.

Symptom reporting--Women report chest pains relatively more frequently than men, but pattern of treatment with medications is similar.

##### STATISTICAL SCIENCES

First use of mixed effects model estimates of rate of change used in risk factor analyses of pulmonary function.

Methodology developed to estimate when significant change in risk factor occurs prior to occurrence of outcome.

Application of mixed effects models to longitudinal studies of aging presented at NIH conference on statistical methodology.

Beginning in August, 1993 the Chief, LSB and ASD for the BLSA will be on a year long work study program at the Eindhoven University of Technology, Eindhoven, the Netherlands. During that time the Scientific Director, NIA, will serve as Acting ASD for the BLSA, and the Medical Officer of the BLSA will serve as Chief, LSB.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00015-35 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Baltimore Longitudinal Study of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Fozard	Assoc. Scientific Director, BLSA	OSD, NIA
L.J. Brant	Mathematical Statistician	LSB, NIA
E.J. Metter	Medical Officer	LSB, NIA
B. Hurley	IPA	UM
C. Morrell	IPA	LC
J.D. Pearson	Senior Staff Fellow	LSB, NIA
B.S. Hiscock	Program Analyst	LSB, NIA

Other Investigators: See next page.

COOPERATING UNITS (if any)

Francis Scott Key Medical Center (FSKMC); National Institute of Dental Research (NIDR); University of Maryland, College Park (UM); Loyola College, Baltimore (LC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.15

PROFESSIONAL:

.80

OTHER:

1.35

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The Baltimore Longitudinal Study of Aging (BLSA), the NIA's major research program on human aging, has been conducted at the Gerontology Research Center since 1958. The overall scientific goals of the BLSA are: 1) To identify differences among individuals of different ages and changes that occur in the serial observations of these individuals with the passage of time; to determine the relative contribution of aging, disease processes, cohort effects and secular effects in producing observed differences and changes; and to establish the degree of interrelation and/or interaction among these factors. 2) To expand scientific understanding about predictors and risk factors for specific diseases and for other end points related to successes and failures of adaptation to aging processes. The BLSA consists of a series of longitudinal and cross-sectional studies of varying degrees of interrelationships oriented toward description, prediction and intervention in human aging processes.

Scientists working with BLSA are assigned to 11 sections of 7 laboratories in addition to the LSB. The Chief, LSB, is the Associate Scientific Director, NIA for the BLSA and LSB staff administer and manage the BLSA as well as conduct research with it.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00624-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Baltimore Longitudinal Study of Aging (BLSA): Population Dynamics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Fozard	Chief & Assoc. Scientific Director	LSB, NIA
	L.J. Brant	Mathematical Statistician	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA
	L.M. Whetstone	Psychologist	LSB, NIA
	C.L. Dent	Supervisory Biologist	LSB, NIA
	C.B. Willey	Program Assistant	LSB, NIA

COOPERATING UNITS (if any)

Francis Scott Key Medical Center

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

1.8

OTHER:

2.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is concerned with optimal management and scientific description of the total BLSA population, which includes, as of 7/8/93, 1173 active participants (508 women; 665 men), 485 inactive (171 women; 314 men), and 580 deceased (54 women; 526 men). Active participants range in age from 20 to 97 years old.

A major effort this year has been recruitment of women and African Americans to meet recruitment goals set in March, 1992, to meet the needs of the two new research initiatives, the Perimenopausal and Vascular studies, discussed in last year's report. At present, 7% of all active participants, and 10% of the active women, are African American. When current recruitment targets are met at the end of FY94, the sample is expected to be 20% African American, and 50% female. Since April, 1992, all new participants have been screened through self report applicant health status questionnaire according to health criteria for either the Vascular or Perimenopausal initiatives.

A most challenging and essential aspect of participant management is tracking of inactive participants to keep up with outcomes important in longitudinal studies, e.g., morbidity, mortality, or changed circumstances which indicate the possibility of re-enrollment as an active participant. In the BLSA, telephone follow-up of all consenting inactive participants (91% of the eligibles) was completed in December, 1992, and the data are now being analyzed. These data are available for all BLSA investigators. We now know the status of 98% of the total number of participants ever studied. A major goal for the coming year is to develop a plan and obtain the resources to maintain this project on an ongoing basis.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00625-04 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Baltimore Longitudinal Study of Aging (BLSA) Data Management

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. A. Shefrin	Computer Scientist	LSB, NIA
Others:	C. B. Eames	Programmer/Analyst	LSB, NIA
	N. S. Gittings	Programmer/Analyst	LSB, NIA
	G. S. Hammen	Computer Technician	LSB, NIA
	S. M. Pegram	Computer Technician	LSB, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center (GRC), Longitudinal Studies Branch (LSB)

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.8

PROFESSIONAL:

2.8

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Data Management work group is responsible for the storage of both paper and computer records generated by the BLSA. They perform the data entry of medical records and manage the data entry of many of the other data collected by the BLSA internal investigators and outside collaborators. Staff members manage the BLSA Computer System and its data base. They support both the administration of the BLSA as well as its scientific activities. Their functions include data extraction, processing, and analysis; consultation; training; hardware and software maintenance; and software development.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00622-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health Disease Status in the BLSA: Clinical Health Evaluation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.J. Metter	Medical Officer	LSB, NIA
J.L. Fozard	Chief	LSB, NIA
B.S. Hiscock	Program Analyst	LSB, NIA
J.L. Fleg	Staff Cardiologist	LCS, NIA
D. Kramer	Nurse Practitioner	FSKMC
D. Binckley	Nurse Practitioner	FSKMC
A. Rosenberg	Nurse Practitioner	FSKMC
C. Kopac	Nurse Practitioner	FSKMC
C. Bacal	Physician Assistant	FSKMC
L. Wherstone	Psychologist	LSB, NIA

COOPERATING UNITS (if any)

Francis Scott Key Medical Center (FSKMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.4

PROFESSIONAL:

.4

OTHER:

0

CHECK APPROPRIATE BOXES

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The health questionnaire implemented on March 1, 1991 was modified so that on subsequent visits the participants are queried regarding changes in their health status since their last visit. The interval history questionnaire was started on March 1, 1993. With the new interval questionnaire, we also changed the branching questions for positive responses to include queries about the effects of symptoms and problems on life style and quality of life. Its goal is to identify how a symptom affects the life of the subject. A physical functioning inventory has been developed and has been implemented into the clinical evaluation that will probe for mild to moderate disability. Over the past year, we have added to our quality assurance program to assess the value of the new health questionnaire for BLSA research. The program had included the development by the nurse practitioners/physician assistants of formal guideline for the health questionnaires, and regular QA meetings to discuss issues pertinent to the clinical evaluation. We have begun an analysis of several clinical questions regarding neck, arm, back and leg pain in order to determine whether the question format and the branching questions can be used to understand the role of symptom complaints in aging. This year we have started to administer the Physical Functioning Inventory to all active participants.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00626-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Changes in Visual Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Fozard	Chief	LSB, NIA
E.J. Metter	Medical Officer	LSB, NIA
N.S. Gittings	Computer Programmer	LSB, NIA
C.L. Dent	Testing Manager	LSB, NIA
F. Schieber	Guest Researcher	USD
D.W. Kline	Guest Researcher	UC
T.S. Kline	Guest Researcher	UC
H.A. Quigley	Investigator	JHU
E.I. Traboulsi	Investigator	JHU

COOPERATING UNITS (if any)

University of South Dakota (USD)

University of Calgary (UC)

The Wilmer Eye Institute (JHU)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.26

PROFESSIONAL:

1.16

OTHER:

.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Visual acuity and binocular depth perception is measured on first time participants, and longitudinally on women participants in the BLSA. A laboratory based assessment of visual contrast sensitivity continues to be administered, increasing the number of persons with at least two measures to over a hundred. A new study of the relationship between intraocular pressure and systemic blood pressure has been developed. Changes in the sensitivity of the visual fields are used to measure the effects of elevated intraocular pressure on visual sensitivity; the intraocular pressure is related in turn to changes in systemic blood pressure. The study will identify possible racial and sex differences in blood pressure/intraocular pressure relationships.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00627-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Risk Factors for Age-Related Ocular Change

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sheila West	Associate Professor	Wilmer Institute JHU
		Guest Researcher	LSB NIA
Other:	Neil Bressler	Associate Professor	Wilmer Institute JHU
	Harry Quigley	Professor	Wilmer Institute JHU
	Evan Farmer	Professor, Dermatopathology	Wilmer Institute JHU
	Susan Vitale	Assistant Professor	Wilmer Institute JHU

COOPERATING UNITS (if any)

Wilmer Institute (Johns Hopkins University School of Medicine)  
National Eye Institute

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine risk factors for the leading causes of blindness in the United States, age-related macular degeneration, cataracts, and glaucoma. Specifically, the study is examining the association of dermal elastotic degeneration and antioxidant vitamin status with age-related macular degeneration; the association of vitamin intake with cataract. A total of 719 participants age 40 and older with at least one visit prior to the ocular study were eligible, of whom 96% had macular and lens photographs to assess ocular status.

Low levels of serum vitamin E status were a risk factor for nuclear opacities. No other measures of antioxidant status, nor a combined antioxidant index, were associated with cataract.

No evidence of increased risks of either macular degeneration or high risk macular characteristics were found associated with elastotic degeneration. Females were significantly less likely to have high risk macular characteristics compared to males.

Low levels of serum vitamin E status were a risk factor for age-related maculopathy, as was low antioxidant status.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00628-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Aging and Auditory Characteristics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sandra Gordon-Salant	Guest Researcher	LSB, NIA
James L. Fozard	Chief	LSB, NIA
E. Jeffrey Metter	Medical Officer	LSB, NIA
Larry J. Brant	Mathematical Statistician	LSB, NIA
Jay D. Pearson	Senior Staff Fellow	LSB, NIA

COOPERATING UNITS (if any)

University of Maryland

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.77

PROFESSIONAL:

.27

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project aims to combine assessment of hearing abilities among subjects of different ages over time, together with information from their communication and health histories. Medical and cognitive data collected from subjects in the longitudinal study will be examined with respect to the audiologic and case history data. The two principal objectives of this project are: A) To study the contribution of medical, genetic, dietary and social factors to age-related auditory dysfunction; and B) To determine to what extent age, independent of other etiologic factors, causes a deterioration in hearing abilities. During the past year, approximately 450 subjects from the BLSA have been tested on all of the new measures in the hearing protocol. These measures include assessment of pure-tone hearing sensitivity, sentence understanding in noise, self-perceived hearing handicap, tympanometry, acoustic reflex thresholds, acoustic reflex magnitude, acoustic reflex adaptation, and acoustic reflex latency (the last five measures are part of the acoustic immittance battery of electrophysiologic tests). We have pursued two types of retrospective analyses. In the first analysis, we are examining risk factors for apparent age-related hearing loss in men and women participants in the BLSA. Subjects included in this analysis exhibited significant deterioration in hearing during the course of data collection, but had no known cause for hearing impairment. Of the three modifiable risk factors examined, only blood pressure had an association with hearing loss, independent of age, in the women. In the second analysis, we are comparing longitudinal changes in hearing thresholds with age among men, women, and men with apparent noise-induced hearing loss (on the basis of a notched audiometric configuration). We plan to develop and distribute a noise exposure questionnaire to these participants in order to identify important sources of incidental noise exposure in this population of predominantly white collar workers.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00635-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Changes in Response Speed and Nerve Conduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Fozard	Chief	LSB, NIA
E.J. Metter	Medical Officer	LSB, NIA
J.L. Wood	Psychologist	LSB, NIA
M. Vercruyssen	Consultant	UH

COOPERATING UNITS (if any)

University of Hawaii (UH)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.47

PROFESSIONAL:

.17

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three measures of slowing of behavior were analyzed to describe age-related differences as well as age changes: reaction time, movement time, and nerve conduction velocity. Age-related changes in reaction time were not as robust as the cross-sectional age differences suggesting that factors other than age are responsible for part of the age declines observed. Reciprocal manual movement speed declines relatively more rapidly with greater task difficulty in older age. Although cautiously reported, nerve conduction velocity decreased with older age with age difference becoming apparent around age fifty.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG-00629-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA Men: Distribution of Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
	L.J. Brant	Mathematical Statistician	LSB, NIA
	J.D. Pearson	Senior Staff Fellow	LSB, NIA
	G. Baker	Guest Researcher	LSB, NIA
	R. Kriner	Consultant	AARP

COOPERATING UNITS (if any)

American Association of Retired Persons (AARP)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NO REPORT FOR THIS PROJECT THIS FISCAL YEAR.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00630-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA Women: Distribution of Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	D. Kramer	Nurse Practitioner	FSKMC
	D. Binckley	Nurse Practitioner	FSKMC
	A. Rosenberg	Nurse Practitioner	FSKMC
	C. Kopac	Nurse Practitioner	FSKMC
	C. Bacal	Physician Assistant	FSKMC

COOPERATING UNITS (if any)

Francis Scott Key Medical Center (FSKMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.21

PROFESSIONAL:

.05

OTHER:

.16

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Aging women experience life changes differently than men. Over the past year, data from the BLSA were analyzed to compare gender similarities and differences (1) in drug treatment of hypertension, (2) symptom reporting with the study of chest pain and its association with heart disease, and differences in the reporting of musculoskeletal pains, and (3) the prevalence of urinary stress incontinence in women and its relationship to the aging process. In the past year slow progress has been made in these studies. Hypertension treatment was compared in two periods (1978-1982 and 1987-1991). No major differences were found in the treatment of hypertension in either time period, or in the application of new medications as they became available between the two time periods. Chest pain was found to be a common health complaint for both women and men. The prevalence at different ages was different by sex, with approximately 25% of women reporting chest pain by age throughout the adult life-span, while men showed an increase in reporting with increasing age. Women also reported more neck and arm pain, while men reported more back pain. Further analyses are progressing in both of these studies.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00632-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA Men: Perceived Health Status

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA
	J.D. Pearson	Senior Staff Fellow	LSB, NIA
	K. Elliott	Guest Investigator	GMU
	P. Knight	Guest Investigator	GMU
	D. Hiebert	Guest Investigator	GMU

COOPERATING UNITS (if any)

George Mason University (GMU)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.1

PROFESSIONAL:

.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

NO REPORT FOR THIS PROJECT THIS FISCAL YEAR.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00641-01 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Race & Gender Differences in Intracerebral & Carotid Arterial Velocity with Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	C. Early	Neurologist	FSKMC
	M. Unger	Special Volunteer, Engineer	LSB, NIA
		Eindhoven U. of Technology,	LSB, NIA
		The Netherlands	

COOPERATING UNITS (If any)

Francis Scott Key Medical Center;  
Eindhoven University of Technology, The Netherlands

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.21

PROFESSIONAL:

.05

OTHER:

.16

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Over the past year, doppler ultrasound equipment was purchased for part of the BLSA Vascular Initiative. We are currently recruiting a visiting fellow to carry out the program. We have evaluated the doppler ultrasound equipment to determine how well it should be able to make the needed measurements. The technical accuracy of the equipment appears to be adequate for the planned measurements.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00633-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA: The Prostate Gland

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.D. Pearson	Senior Staff Fellow	LSB, NIA	
E.J. Metter	Medical Officer	LSB, NIA	
J.L. Fozard	Chief	LSB, NIA	
L.J. Brant	Statistician	LSB, NIA	
R. Andres	Chief	LCP, NIA	
S.M. Harman	Section Chief	LCP, NIA	
H.A. Guess	Guest Researcher	MSD	
H.B. Carter, A.W. Partin	Assistant Professor	JHU	
P.C. Walsh	Professor	JHU	

COOPERATING UNITS (if any)

Johns Hopkins University (JHU), Department of Urology  
Merck, Sharp & Dome (MSD), Department of Epidemiology

LAB/BRANCH

Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, Gerontology Research Center

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new BLSA study of Prostate Growth and Disease was begun in February 1993 to examine anatomic and physiologic correlates of normal prostate growth and the development and progression of benign prostatic hyperplasia and prostate cancer.

In the past year, four studies have been completed. A BLSA study of clinical markers of poor prognosis from prostate cancer (PCa) found that prostate-specific antigen (PSA) level and PSA velocity (rate of change) prior to diagnosis were not strong predictors of progression to metastasis or death from PCa.

The second BLSA study showed that the epithelial composition of the prostates of men with benign prostatic hyperplasia (BPH) was positively correlated with PSA level and PSA velocity. Thus, PSA could be useful as an inexpensive method of targeting drug treatments at either the epithelial or stromal components of BPH.

The third study developed formulas utilizing PSA level, histologic grade and clinical stage to predict whether the cancer had spread outside the prostatic capsule, into the seminal vesicles, or lymph nodes in 703 patients from Johns Hopkins Hospital. Clinicians will use the formulas to guide treatment decisions.

The fourth study examined clinical markers of local or distant recurrence of prostate cancer in men who have undergone radical prostatectomy. PSA level, PSA velocity, Gleason score, and pathologic stage were assessed among a clinical series from Johns Hopkins Hospital of 51 radical prostatectomy patients who had either a local or distant recurrence of PCa. PSA velocity was the strongest predictor of type of recurrence, along with Gleason Score and pathologic stage. These findings suggest that PSA velocity is an important clinical tool for guiding treatment of recurrences of prostate cancer after radical prostatectomy.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00634-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Changes in Pulmonary Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Melvyn S. Tockman	Guest Researcher	LSB, NIA
Other:	Jerome L. Fleg	Senior Staff Cardiologist	LCS, NIA
	James L. Fozard	Chief	LSB, NIA
	E. Jeffrey Metter	Medical Officer	LSB, NIA
	Jay D. Pearson	Senior Staff Fellow	LSB, NIA

COOPERATING UNITS (if any)

The Johns Hopkins School of Hygiene  
The Johns Hopkins School of Medicine

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.16

PROFESSIONAL:

.31

OTHER:

.85

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The GRC-BLSA Program in Pulmonary Aging focused upon:

a. Development of a Mathematical Model of Pulmonary Aging

A new model of normal pulmonary aging is being developed based upon the change in physiologic emptying of the lungs. Digitized spirometry from BLSA healthy, nonsmokers, without evidence of heart disease, is converted into distributions of emptying times by moments analysis. Those healthy nonsmokers with minimal lung function decline will define the standard of optimal pulmonary aging. Aging of the lung will be defined as a significant increase beyond optimal in the proportion of ventilatory emptying described by long time constants. The age-related decline of individual pulmonary function may be described over longitudinal follow-up by a mixed-effects model which includes parameters for Intercept, Time Interval, (Time Interval)<sup>2</sup>, Age and Mean Emptying Time.

b. Accelerated Decline in Pulmonary Function Predicts Coronary Heart Disease

An accentuated risk for cardiac death follows a large decline in FEV<sub>1</sub>, independent of the effects of the initial FEV<sub>1</sub>% Predicted, cigarette smoking and other common CHD risk factors. There were 79 cases of CHD death and 804 survivors over the 1 to 28.5 year follow-up period. At study entry, cases were older, had a lower FEV<sub>1</sub>, a higher cholesterol level, a higher prevalence of hypertension and cigarette smoking. After adjusting for these factors in a Cox linear effects model, the most striking relative risks for cardiac mortality were associated with quintile of subsequent FEV<sub>1</sub> decline. FEV<sub>1</sub> decline increases with age, but at any age, successive increasing quintiles of FEV<sub>1</sub> loss experienced 1.00, 2.92, 4.49, 5.13, and 3.27-fold excess risk of cardiac death. Significant association has been observed between rate of pulmonary function decline and the development of IHD.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00640-01 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Age on Muscle Strength, Body Composition and Health Status

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ben Hurley	Guest Researcher	UM
	Rosemary Lindle	IRTA Fellow	LSB, NIA
Others:	James L. Fozard	Chief	LSB, NIA
	E. Jeffrey Metter	Medical Officer	LSB, NIA
	Jerome L. Fleg	Sr. Staff Cardiologist	LCS, NIA

COOPERATING UNITS (if any)

Dept. of Kinesiology, College of Health & Human Performance, University of Maryland (UM), College Park

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.67

PROFESSIONAL:

1.67

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

This study examines the natural course of age-related changes in muscular strength and determines the relationship of age and strength levels to body composition and health status. The relationship of age to maximal force production (strength) of the upper and lower body musculature during the concentric (shortening) and eccentric (elongation) phases of movement are presently being established. In addition, strength measures are being assessed at slow, fast and zero (isometric) speeds to determine if there is a preferential loss of force at fast speeds with age. This information will be related to the selective atrophy of type II fibers that occurs with age. Analyses of changes in force production in the prime mover is being compared to changes in the antagonist muscle group to determine if muscle balance is affected with age. In addition, the angle of greatest force production is being assessed to determine if this angle changes with age. New testing equipment has been purchased and a new protocol has been established for this project. Over 200 male and female subjects from the 20s through the 80s have been tested so far this year.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00636-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Physical Activities in the BLSA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Linda P. Fried Guest Researcher LSB NIA

Other: Jerome L. Fleg Cardiologist LCS NIA  
E. Gundy Zenger Research Assistant JHU  
Jordan D. Tobin Chief, Applied Physiology LCP NIA  
James L. Fozard Chief LSB NIA

COOPERATING UNITS (If any)

Johns Hopkins Medical Institutions

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NO REPORT FOR THIS PROJECT THIS FISCAL YEAR



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00639-02 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Individual Changes in Functioning with Age and Target Conditions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Lois M. Verbrugge	Guest Researcher	LSB, NIA
	Ann L. Gruber-Baldini	Guest Researcher	LSB, NIA
Others:	E. Jeffrey Metter	Medical Officer	LSB, NIA
	Jay D. Pearson	Senior Staff Fellow	LSB, NIA
	Larry Brant	Mathematical Statistician	LSB, NIA
	Cathy Dent	Supervisory Biologist	LSB, NIA
	James L. Fozard	Chief	LSB, NIA

COOPERATING UNITS (if any)

Institute of Gerontology, University of Michigan

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The principal research focus is to analyze longitudinal changes in functioning among BLSA subjects. The aims are to characterize intra-individual changes in 14 domains of activity and to study how these changes vary by sociodemographic and medical factors. The data for these analyses are derived from the "Activity Questionnaire II." It has been filled out by BLSA subjects at each visit since 1966. Subjects estimate the amount of time they spend on numerous specific activities, ranging from personal care to leisure. This data set is unique for its time stretch (up to 25 years for some subjects) and its content (the comprehensive scope of activities). Analyses on this data set include examinations of the cross-sectional, longitudinal, and secular patterns by age and gender. Cross-sectional analyses reveal consistent age and gender differences for participation in and time spent doing various activities, especially work, housework, childcare, and various discretionary activities. Comparisons of longitudinal and cross-sectional results show evidence of secular changes in time spent doing work, housework, and childcare by women. Examination of the effect of chronic conditions on time spent in activities revealed that the presence of a chronic condition (diabetes, hearing loss, hypertension, ischemic heart disease, musculoskeletal problems, pulmonary dysfunction, and visual acuity problems) increases the time spent in obligatory activities (personal care, sleep) and decreases time in discretionary activities (socializing, public service). The effect of a chronic condition on committed activities (housework, childcare) interacted with gender so that women increased time in these activities while men decreased their time; this may be a function of gender differences in perception of commitment of activities (e.g., women being more committed to housework).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00637-04 LSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gender Differences and Individual Variability in Human Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.J. Brant	Mathematical Statistician	LSB, NIA
	C.D. Pearson	Senior Staff Fellow	LSB, NIA
	C.H. Morrell	Guest Worker	LSB, NIA
Others:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer, BLSA	LSB, NIA

COOPERATING UNITS (if any)

Laboratory of Cardiovascular Sciences, NIA (J.L. Fleg)  
Speech and Hearing Sciences, University of Maryland (S. Gordon-Salant)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of gender differences and individual variability in age-related phenomena are being carried out to: 1) determine the "normal" range of variability in human aging, 2) identify potential sources of variability which may be responsive to intervention, and 3) determine if there are subgroups of individuals who are more susceptible or resistant to various aspects of aging. The research combines the use of sophisticated statistical methodologies and the unique time depth and multidisciplinary breadth of the existing BLSA data base to examine issues related to the concepts of "normal" and "successful" aging, as well as to increase the power of traditional research designs. The statistical methods used include longitudinal regression models, time dependent proportional hazards analysis, and finite mixture models. Major findings include: 1) hearing sensitivity--there is a gender cross-over in hearing thresholds with men have better sensitivity at low frequencies and women having better sensitivity at high frequencies; 2) hearing change--longitudinal rates of decline in hearing sensitivity are at least twice as fast in men compared to women; 3) blood pressure--BLSA men and women do not exhibit the commonly observed gender cross-over in blood pressure with increasing age; 4) blood pressure change--preliminary findings suggest that accelerated longitudinal changes in blood pressure are a risk factor for coronary events; and 5) pulmonary function--longitudinal rates of decline in pulmonary function are faster in never-smoking men than in women. These findings represent significant contributions to the theoretical and methodological development of biomedical risk factor studies, as well as to an increased understanding of the dynamics of the aging process. Research is underway to develop more refined methods of studying variability in aging in order to develop theoretically and methodologically sound approaches to risk factor analysis which account for changes in an individual's covariates over time and the possibility that individuals differ in susceptibility or resistance to aging processes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00638-04 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health Promotion, Modifiable Risk Factors and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L.J. Brant Mathematical Statistician LSB, NIA

Others: J.L. Fozard Chief LSB, NIA

E.J. Metter Medical Officer, BLSA LSB, NIA

J.D. Pearson Senior Staff Fellow LSB, NIA

COOPERATING UNITS (if any)

Department of Urology, Johns Hopkins School of Medicine (HB Carter)  
Speech and Hearing Sciences, University of Maryland (S Gordon-Salant)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.25

PROFESSIONAL:

0.1

OTHER:

0.15

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Unnecessary morbidity and mortality is an important problem which leads to increased health-care costs and can ultimately result in premature death. It has been estimated that approximately two thirds of mortality is due to potentially preventable causes - 1.2 million deaths (65%) and 8.4 million years of life lost before age 65 (63%). Principal factors associated with unnecessary morbidity and mortality include tobacco use, high blood pressure, improper nutrition, lack of screening and prevention services, alcohol abuse, and injury. This project uses longitudinal data from the Baltimore Longitudinal Study of Aging (BLSA) and other studies to examine the influence of modifiable risk factors on the occurrence of premature deaths and unnecessary morbidity and disability. Identification of risk factors can lead to primary prevention efforts. Examples of BLSA research on the identification of modifiable risk factors include: noise exposure, blood pressure, and smoking in relation to hearing loss; pulmonary change as a risk factor for cardiac events (see Project Z01 AG 00634-04 LSB); and hormonal factors as a risk factor for prostate disease (see Project Z01 AG 00633-03 LSB). One example of BLSA research on secondary prevention practices is the ongoing research to improve the accuracy of the prostate specific antigen test to allow screening for early detection of prostate cancer (see also Project Z01 AG 00633-03 LSB). Information from these studies can have an impact on the development of primary and secondary prevention programs to improve longevity and quality of life for many Americans.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00623-05 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Statistical Methodology for the Analysis of Studies of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L.J. Brant Mathematical Statistician LSB, NIA

Others: J.D. Pearson Senior Staff Fellow LSB, NIA  
C.H. Morrell Guest Worker LSB, NIA

COOPERATING UNITS (if any)

Department of Mathematical Sciences, Loyola College in Maryland (C.H. Morrell)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Statistical methodology is being applied and developed for longitudinal studies and other studies of aging. The research program focuses on several types of statistical models: 1) longitudinal mixed-effects regression models which consider both within- and between-subject variation in analyzing the repeated measurements for all individuals in the study population, 2) survival analysis for studying risk factors in prospective studies, 3) multiple comparisons for testing group differences in experimental or observational designs, 4) mixture models for describing age changes in distributions of biological markers, and 5) experimental design. Other techniques used include Bayesian, maximum likelihood and numerical computing methods. A major emphasis of the research program is the development of methods which yield cogent yet easily understood results when applied to data.

Several mixed-effects longitudinal regression analyses have been completed. An analysis of longitudinal changes in hearing sensitivity in BLSA men and women was the largest mixed-effects analysis ever reported. A method of estimating when an event took place (e.g., first detectable PSA increase from prostate cancer) was developed using a piecewise nonlinear mixed-effects analysis. A new method of examining longitudinal change as a risk factor in survival analysis was developed using a two-stage time-dependent Cox proportional hazards regression approach.

The research program has extended earlier methods of longitudinal data analysis, introduced novel methods of describing the natural history of aging, and developed new approaches toward the use of longitudinal data in epidemiological and biomedical studies of aging and associated disease states.



LCS

LCMB

LCP

LBS

LBC



## ANNUAL REPORT OF THE LABORATORY OF BEHAVIORAL SCIENCES

### NATIONAL INSTITUTE ON AGING

#### Significant Administrative Events

There have been a number of personnel changes within this fiscal year. Dr. Sergei Kirov and Dr. Svetlana Shefer have joined the Behavioral Physiology Section as Visiting Fellows. Dr. Kirov will work in the thermoregulation program and Dr. Shefer will be involved in the hemodynamic studies. Dr. Olga Federova has joined the Behavioral Medicine Section as a Visiting Fellow. She will be studying the role of digoxin-like factors in behaviorally-induced rises in blood pressure. Dr. Paul Chew, who was an IPA Fellow in the Behavioral Physiology Section, resigned and took a position as Director of Clinical Research at the Bristol-Myers Squibb Pharmaceutical Research Institute.

The Behavioral Nursing Research Program (reviewed below) includes research programs which are supported, in part, by an intra-agency agreement between NIA and the National Institute on Nursing Research. The agreement supports a Staff Fellow, an IPA Fellow and research assistants.

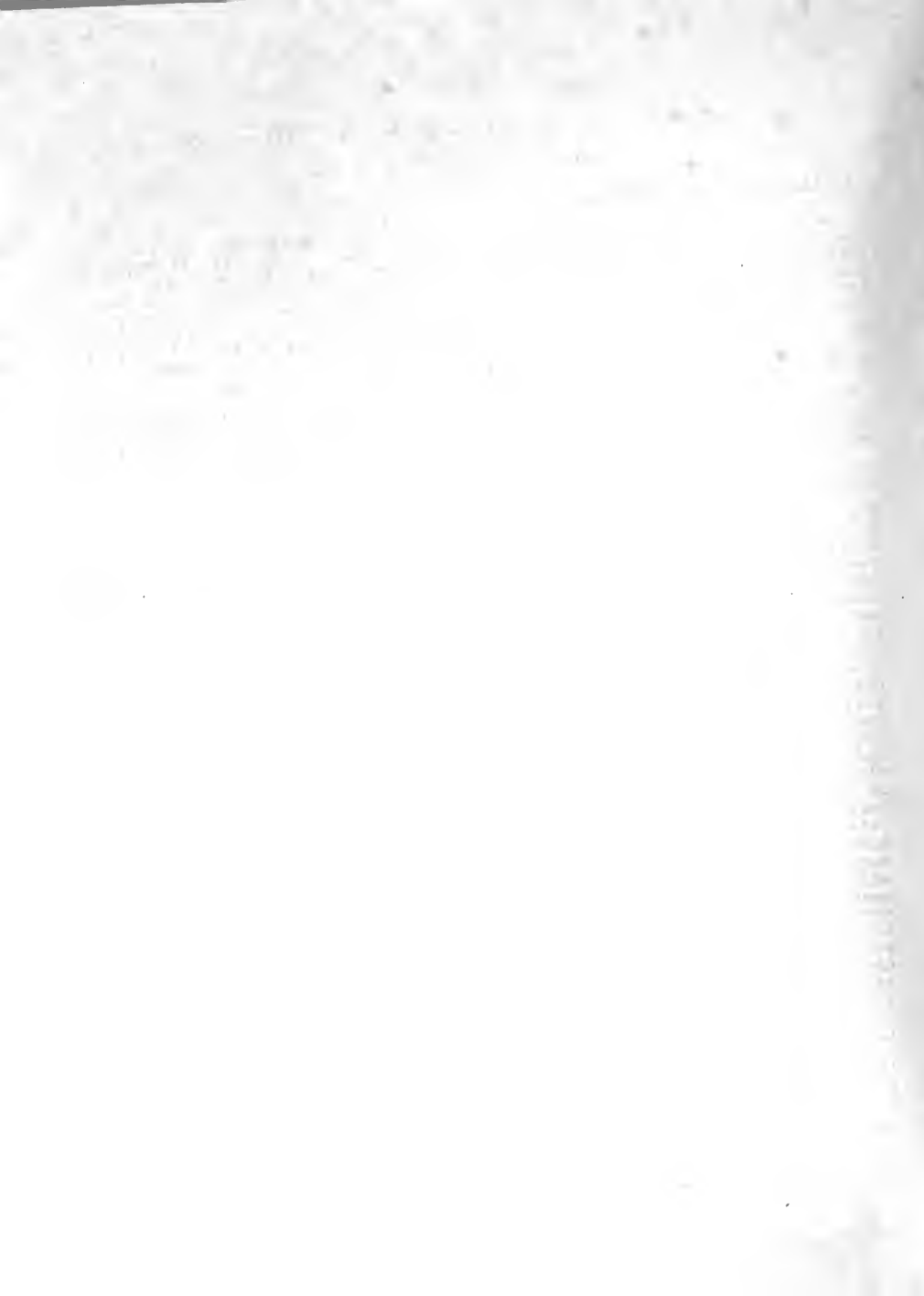
#### Research Advances

##### Nocturnal Hemodynamics

Previous research in our nonhuman primate laboratory showed that there is a characteristic, overnight pattern of change in the circulation: this pattern includes monotonic falls in cardiac output and central venous pressure, and a monotonic rise in peripheral resistance. This pattern results in a reduced, but stable blood pressure throughout the night. Other research has suggested that this hemodynamic pattern is, at least in part, a homeostatic adjustment to an overnight fall in plasma volume: the hematocrit is consistently higher in the morning, and blood viscosity is also greater in the morning. Research during the past year has been designed to characterize some of the mechanisms mediating these effects, and to identify clinically significant features of these hemodynamic effects. Studies of mechanism have concentrated on two problems: 1) the characterization of the cardiac-specific factors that mediate cardiac output; and 2) the overnight pattern of change in plasma volume. Findings from our clinical study will be summarized below. In order to evaluate cardiac function in greater detail, we have been conducting periodic echo-Doppler studies of overnight changes in various cardiac parameters of filling -- e.g., iso-volumic relaxation time, end-diastolic volume, and emptying: e.g., stroke volume, ejection fraction, pre-ejection period, velocity of contractile shortening. In general, we have found evidence that in the morning cardiac filling is diminished and cardiac contractility is weaker. These effects seem to persist even when heart rate or central venous pressure is prevented from falling overnight. We are now attempting to develop methods for noninvasively measuring overnight changes in plasma volume; however, these studies have just begun and there are no data yet available. We have completed one clinical study this year. In the course of the studies in which we prevented heart rate from falling overnight, we observed significantly poorer cardiac function, especially in the morning. In a study we just completed, we have seen similar effects in patients who have pacemakers chronically implanted in their hearts. Our findings revealed that when the heart is paced for 3 weeks at 80 beats/min (the upper limit of normal), cardiac function is poorer than when the heart is paced for 3 weeks at 50 beats/min (lower limit of normal). Furthermore, the effect of pacing rate is exacerbated in the morning relative to the evening.

##### Thermoregulation

Elderly persons are at risk to develop cardiac dysfunctions in response to cold stress. This risk is enhanced when the subject exercises in the cold. Two studies using animal models are designed to identify some of the mediating mechanisms involved in this phenomenon. One study has shown that there is an



increase in sympathetic nervous activity to brown adipose tissue in old C57BL/6J mice relative to adult animals: the increase in sympathetic activity is comparable to that seen in adult animals which are cold-adapted. These findings indicate that the old animals readily sense the cold and react to it. However, they neither raise nor maintain their body temperatures as much as do the adult animals suggesting either a defect in heat production or heat conservation, or both. Current research is designed to analyze this defect in greater detail. Studies of the interaction between cold tolerance and physical training have just begun. Preliminary findings suggest: 1) Adult animals which exercise and then are confronted with a cold stress show a decrease in cold tolerance; however, this effect can be eliminated in old animals by gradually introducing them to the exercise; 2) there are diurnal differences in cold tolerance in adult and aged mice: mice, which are nocturnal animals, have poorer cold tolerance and lower metabolic heat production in the afternoon. Exercise, prior to a cold test will reduce the diurnal differences in cold tolerance, but not the differences in metabolic heat production. This finding suggests that heat conservation may be poorer during the period of circadian rest, and that exercise may "prime" the vasculature to respond more effectively to the cold stress at this time.

#### Blood Pressure Regulation

The main focus of the research in this program is to identify the physiological mechanisms that are responsible for elevations in blood pressure levels in salt-loaded subjects during aversive behavioral conditions. Previous research had shown that dogs, preceding the onset of avoidance tasks, and humans in work and social environments, emit an inhibitory breathing pattern characterized by slow rate and normal tidal volume. This inhibitory breathing pattern is associated with a sustained decrease in plasma pH and a progressive increase in plasma bicarbonate concentrations and  $\text{pCO}_2$  as well as an increase in blood pressure and decrease in heart rate. Dogs who are salt-loaded in the aversive setting can develop severe hypertension; and medical students who are preparing for an important examination, when they ingest higher than normal amounts of salt, show mild, sustained elevations of blood pressure that are not present in classmates on a normal sodium intake, nor is the salt diet sufficient in the students when no examination is imminent. Laboratory studies carried out this year have shown that 30 minutes of inhibitory breathing increases renal reabsorption of sodium, but not potassium. In addition, urinary levels of a digoxin-like factor which is known to inhibit sodium-potassium-ATPase activity and to increase intravascular sodium, also occurred during the inhibitory breathing task. Taken together, these findings suggest that inhibitory breathing which occurs during aversive behavioral conditioning and which would increase vascular sensitivity to sodium intake, could mediate a sustained rise in blood pressure. An additional study completed this year showed that resting, transthoracically measured  $\text{pCO}_2$  is higher in subjects who typically have higher resting blood pressures, and in black subjects relative to white subjects.

#### Behavioral Nursing Research Program

The extraordinary rise in health care costs over the past 25 years is, in part, the result of chronic disabilities among the elderly. One major disability is hip fractures which affects 270,000 persons each year and accounts for \$7 billion in health care costs. A second major disability is incontinence which affects 10 million persons and accounts for \$10 billion in health care costs. Research in this program addresses both of these problems. A recently completed, retrospective medical record review of 50, consecutive hospital admissions for hip fracture (average age, 77 years), revealed that 35% of the patients were discharged to nursing homes and 4% had died; 32% were incontinent of urine, and 33% required catheterization for urinary retention. Among the 61% who were discharged to home, 27% required a wheelchair and 73% used a walker; 47% were non-weight-bearing, 10% were partial weight-bearing and 40% were weight-bearing. Future research is designed to assess the effectiveness of nursing interventions, beginning during the hospitalization phase and continuing to the home to see if some of these adverse and costly effects of hip fracture can be ameliorated through a program of early mobilization which appears to be a major factor in





rehabilitation and recovery.

Previous research in LBS has shown that many community-living, incontinent patients can be taught to regain partial or full control over their bowel or bladder function. Other research has revealed that incontinent, nursing home residents also can benefit from behavioral interventions. However, in contrast to the community-living situation, in the case of the nursing home, much of the intervention must be directed at improving staff care. The reason staff management programs are important is that most nursing home residents are too disabled to toilet themselves. Earlier research in LBS had shown that the nursing staff in a hospital-based nursing home could be trained to improve continence care, and to reduce incontinence. Subsequent research showed how to develop an effective staff management program for a community-based nursing home. Current research, being carried out by a scientist from NINR, is designed to establish a continence program that can be entirely maintained and operated by indigenous staff in a nursing home. If this program is successful, it will result in a significant advance in nursing home care for three reasons: first, it will enable the incontinent, nursing home resident to live out her life at a higher level of personal dignity; second, it will provide a model for nursing homes to meet the continence care regulations specified by the Health Care Financing Administration; and finally, it will result in a significant cost savings in long-term care since incontinence is known to increase the risk for serious and costly medical complications.

A collaborative study with The Johns Hopkins Division of General Obstetrics and Gynecology was recently implemented to investigate the effectiveness of combined behavioral treatment (pelvic muscle exercises -- PME) and topical estrogen in the treatment of urinary urgency and frequency in post-menopausal women. Urgency and frequency are prevalent in post-menopausal women and significantly impact on their quality of life. Previous research in LBS has shown that biofeedback training including PME can be effective in treating stress incontinence and urge incontinence; however, we have never systematically studied urgency and frequency, per se. Topical estrogen therapy has been used in post-menopausal women to improve vaginal muscle tone; however, there are no known systematic studies of its effectiveness in the treatment of urgency and frequency. It is expected that the combination of PME and topical estrogen will reduce these vexing symptoms.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00063-25 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Learned Modification of Visceral Functions in Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA

OTHERS: Mark I. Talan, Medical Officer LBS, NIA  
Paul H. Chew, IPA LBS, NIA  
David L. Bush, Special Volunteer LBS, NIA  
Robert T. Abel, Special Volunteer LBS, NIA  
Svetlana Shefer, Visiting Fellow LBS, NIA

COOPERATING UNITS (If any)

The Johns Hopkins University Medical School, Francis Scott Key Medical Center,  
Division of Cardiology, Baltimore, MD (P.H. Chew, D.L. Bush, R.T. Abel)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project investigates the role of the central nervous system in the regulation of the circulation. Instrumental conditioning is used to directly modify cardiovascular responses. Naturally-occurring, adaptive responses are examined by monitoring overnight changes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AG 00072-07 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Behavioral Assessment and Treatment of Incontinence in Nursing Home Residents**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bernard T. Engel, Chief

LBS, NIA

OTHERS: Mary H. Palmer, Staff Fellow

CTL, NCNR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Terminated. (See Annual Report of the NINR, Project Number Z01 NR 00004-01 CTL.)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00073-05 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiology of Thermoregulation and Aging in Rodents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mark I. Talan, Medical Officer (Research) LBS, NIA

OTHERS: Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA  
Orit Shechtman, Visiting Associate LBS, NIA  
Sergey Kirov, Visiting Fellow LBS, NIA

COOPERATING UNITS (if any)

Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (A. Sato, Y. Sato);  
Molecular Neurobiology Unit, NIA (G. Higgins)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

3.55

PROFESSIONAL:

2.50

OTHER:

1.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is (1) to investigate age-related changes in thermoregulation, and (2) to examine the physiological mechanisms underlying these changes.

We have demonstrated that aged mice have diminished cold tolerance and are not able to adapt to repeated cold exposure. The cause of these age-related aberrations in thermoregulation appears to be, in part, a reduction in metabolic heat production due to change in brown adipose tissue (BAT) and, in part, a reduction in heat conservation.

Efferent sympathetic nervous responses to BAT are enhanced in both cold-acclimated and aged animals, which suggests that the sympathetic nervous system plays a major role in cold acclimation, but is not responsible for the aged-related decline in thermoregulation. Our results show that sympathetic nervous activity increases with age to adapt to age-related declines in effector organ function.

We have shown the existence of diurnal differences in cold tolerance and metabolic heat production of adult and aged mice: in the afternoon the cold tolerance is poorer and metabolic heat production during cold exposure is lower. One hour of mild exercise prior to cold exposure attenuated diurnal differences in cold tolerance but not in metabolic heat production.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00600-05 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Respiratory Factors in Blood Pressure Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: David E. Anderson, Chief, Behavioral Physiology Section LBS, NIA

OTHERS: Alexei Y. Bagrov, Visiting Associate LBS, NIA  
Olga Fedorova, Visiting Fellow LBS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

2.45

PROFESSIONAL:

1.4

OTHER:

1.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The development of hypertension is potentiated by synergistic interactions between high sodium intake and behavioral factors, but the mediating physiological mechanisms remain to be clarified. An animal model of hypertension suggests that sodium sensitivity may be influenced by a hypoventilatory breathing pattern evoked by aversive conditioning procedures. Previous studies with an ambulatory respiration monitor have shown that episodes of low frequency/normal tidal volume breathing can be observed, in humans in the natural environment. Ongoing laboratory studies with human subjects are showing that the inhibitory breathing pattern is accompanied by increases in  $pCO_2$ , decreases in pH, increases in renal sodium reabsorption, increases in urinary excretion of endogenous digitalis-like factors, decreases in sodium pump activity, and increases in blood pressure, but not heart rate. This physiological pattern provides an alternative to the sympathetic nervous system as a mechanism whereby behavioral factors may interact with high sodium intake to produce sustained hypertension in laboratory animals and humans. These studies may lead to nonpharmacological interventions for prevention and reversal of hypertension and associated disorders of aging.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00603-03 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Implications of Nocturnal Hemodynamic Events

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Paul H. Chew, IPA

LBS, NIA

OTHERS: Bernard T. Engel, Chief

LBS, NIA

Mark I. Talan, Medical Officer (Research)

LBS, NIA

David L. Bush, Special Volunteer

LBS, NIA

COOPERATING UNITS (If any)

Johns Hopkins Univ. Medical School, Francis Scott Key Medical Center, Division of Cardiology, Baltimore, MD 21224 (P. Chew, D. Bush)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.00

PROFESSIONAL:

.70

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in animals have shown significant nocturnal hemodynamic patterns that may contribute to morbid changes in cardiovascular functions in patients with heart diseases. This project is designed to evaluate overnight cardiovascular effects in select patient groups and compare these to control groups.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00604-03

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Behavioral Factors in Blood Pressure Regulation**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: David E. Anderson, Chief, Behavioral Medicine Section LBS, NIA

OTHERS: Jennifer A. Haythornthwaite, Sr. Staff Fellow LBS, NIA

Marilda N. Lipp, Guest Researcher LBS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AG 00606-03 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Behavioral Intervention for Alzheimer's Patients and Their Caregivers**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA

OTHERS: Maria Mannarino, Medical Officer OEA, NIA  
Carol Fuchs-Kinslow, Clinical Social Worker LNS, NIA  
Marie Holly, Clinical Nurse LNS, NIA

COOPERATING UNITS (if any)

Office of Extramural Affairs, NIA (M. Mannarino); Laboratory of Neurosciences, NIA (C. Fuchs-Kinslow; M. Holly)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00607-02 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Effects on Blood Pressure and Circulating Sodium-Pump Inhibitors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: David E. Anderson, Chief, Behavioral Medicine Section LBS, NIA

OTHERS: Alexey Y. Bagrov, Visiting Associate LBS, NIA  
Edward G. Lakatta, Chief, Laboratory of Cardiovascular Science LCS, NIA

COOPERATING UNITS (if any)

University of Maryland School of Medicine, Baltimore, MD (J. Hamlyn)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

.45

PROFESSIONAL:

0.20

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Epidemiological studies have shown that mean blood pressure and the prevalence of hypertension both increase with age. A possible role for dietary sodium intake in the blood pressure increase in aging individuals has been documented, but little information is available concerning possible age-associated changes in circulating hormones that affect sodium transport across vascular smooth muscle. The present study assesses individual differences in levels of two circulating sodium-pump inhibiting hormones as predictors of age-associated changes in resting blood pressure. Morning supine blood pressure and endogenous ouabain and digitalis-like factor are measured in Baltimore Longitudinal Study on Aging participants who meet the inclusion criteria. The study also investigates possible differences in circulating sodium-pump inhibitors as a function of race and gender. Data supporting the hypothesis of a positive correlation between resting blood pressure and circulating sodium-pump inhibitors could lead to pharmacological and nonpharmacological intervention in hypertension focusing on sodium-pump inhibitors and their determinants.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00608-01 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Post-Operative Complications and Mobility Outcomes in Hip Fracture Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ann H. Myers, Senior Staff Fellow

LBS, NIA

OTHERS: Mary H. Palmer, Staff Fellow

CTL, NINR

Bernard T. Engel, Chief, Laboratory of Behavioral Sciences

LCS, NIA

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD; Francis Scott Key Medical Center, Baltimore, MD

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.35

PROFESSIONAL:

1.25

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Hip fractures represent a serious and costly health problem among the elderly. It has been estimated that by the year 2000 there will be 300,000 hospitalizations for hip fractures. Hip fractures account for \$7 billion a year in health care costs. Recovering independence from this serious injury remains a major problem for the elderly. Studies of outcomes suggest that over half of the patients cannot walk independently a year after the injury. Post-operative complications can retard initial rehabilitative efforts. Ambulation status upon discharge is a significant factor associated with post-hospital outcomes. Mobility problems and urinary incontinence often lead to costly institutionalization.

The post-operative complications of pneumonia, decubitus ulcer, urinary retention requiring straight catheterization and urinary incontinence can be reduced by nursing interventions during the acute hospital phase of recovery. Early mobilization probably remains the single most effective method of reducing the incidence of post-operative complications. A majority of patients were discharged home, were nonweight-bearing and needed assistance to ambulate. Behavioral nursing interventions may facilitate rehabilitation in the home and reduce mobility and urinary problems, which can lead to institutionalization.

The findings from this study are being validated by a study of 50 patients hospitalized for hip fracture at an urban community hospital.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00609-01 LBS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sodium Pump Inhibitors in Cardiovascular Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alexei Y. Bagrov, Visiting Associate

LBS, NIA

OTHERS:

Olga Fedorova, Visiting Fellow

LBS, NIA

David E. Anderson, Chief, Behavioral Physiology Section

LBS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.65

PROFESSIONAL:

1.40

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence has accumulated that digitalis-like factors (EDLF) may play an important role in the pathogenesis of cardiovascular disease (hypertension, congestive heart failure, acute myocardial infarction) via their ability to inhibit sodium pump activity. Much attention has been paid to one of these factors, an endogenous ouabain; however, a digoxin-like immunoreactive factor has also been found previously to contribute to arrhythmogenesis in myocardial ischemia. The objectives of this project are to clarify the role of various sodium pump inhibitors, especially the digoxin-like immunoreactive EDLF, in blood pressure regulation.

Over the past year, studies have been performed with instrumented dogs whose plasma volume was expanded via saline infusion. Increases in plasma and urinary EDLF evoked by this procedure were associated with increases in cardiac contractility, urinary sodium excretion, and variable increases in blood pressure. Pretreatment of the animals with an antidigoxin antibody prevented or attenuated these effects. Effects of sodium pump inhibitors on vascular tone and vascular sodium pump activity in rat aortas and dog femoral arteries were studied in vitro. Compared with the effects of ouabain, EDLF obtained from the venom of Bufo marinus toad caused direct inhibition of sodium pump resulting in rapid and sustained vasoconstriction. Unlike ouabain, this EDLF acted independently of the sympathetic nervous system. These studies have implications for understanding of the origins of hypertension and for the development of new pharmacological interventions in cardiovascular disorders.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NR 00004-01 CTL

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Urinary Continence Status and Treatment of Incontinence in Nursing Home Residents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary H. Palmer, Staff Fellow

CTL, NINR

OTHERS: Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD; The John Hopkins Geriatric Center, Baltimore, MD (A. Langford, A. Warwick, S. Denman)

LAB/BRANCH

Laboratory of Behavioral Sciences (Clinical Therapeutics Laboratory, NINR)

SECTION

INSTITUTE AND LOCATION

NIA/NINR, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

0.85

PROFESSIONAL:

0.75

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Urinary incontinence is prevalent in nursing homes. This project is designed to test the effectiveness of staff performance feedback in conjunction with behavioral treatment of incontinence.

This research project is also being reported by the National Institute of Nursing Research.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NR 00006-01 CTL

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Estrogen on Urinary Incontinence and Symptoms in Post-menopausal Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mary H. Palmer, Staff Fellow

CTL, NINR

OTHERS: None

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD; The Johns Hopkins Medical Systems (D. Foster); Francis Scott Key Medical Center, Baltimore, MD (J. Marks).

LAB/BRANCH

Laboratory of Behavioral Sciences (Clinical Therapeutics Laboratory, NINR)

SECTION

INSTITUTE AND LOCATION

NIA/NINR, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

0.40

PROFESSIONAL:

0.30

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Incontinence and urinary symptoms of frequency and urgency are prevalent in post-menopausal women. This project is designed to evaluate the effectiveness of topical estrogen in the behavioral treatment of urinary symptoms and incontinence.

This research project is also being reported by the National Institute of Nursing Research.



LCS

LCMB

ICP

IMG

LBC



## ANNUAL REPORT 1993

### SUMMARY OF THE LABORATORY OF BIOLOGICAL CHEMISTRY

New Themes for the Laboratory of Biological Chemistry--Relevance to Aging and Age-Associated Disorders: The addition of strong programs in cell and neurobiology can lead to important insights into aging, to age-associated diseases and disabilities, and to the development of interventions. Consistent with these themes and with the overall priorities of the Institute, several independent groups have been developed within the Laboratory of Biological Chemistry to investigate age-related changes at the cellular and molecular level. These individual projects are described in detail in the reports that follow. Some of the current activities in the Laboratory are highlighted briefly below.

Review of the Laboratory of Biological Chemistry by the Board of Scientific Counselors, May 11, 1993: As part of the regular review of Intramural Programs by the Board of Scientific Counselors and ad hoc reviewers, the activities of the Laboratory of Biological Chemistry were reviewed. The previous review took place in 1986 when Dr. Bertram Sacktor, now deceased, was Laboratory Chief. As detailed in last year's Annual Report, the Laboratory has undergone a substantial reorganization with reassignment of some staff and recruitment of others. In general, the review was positive about the staff and programs of the Laboratory and endorsed its activities as being appropriate for the mission of the Institute. Various suggestions in the Board's report are being addressed.

Neurobiology: Neurobiology is one of the most active research fronts in biomedical research. The NIA has played an important role in the development of neurobiology research as part of its broad attack on Alzheimer's disease and other forms of dementia. It was our belief that the intramural research program of the Gerontology Research Center would benefit by starting such a program focused on age changes. This program is only a few years old and is currently concentrated on glutamate receptors, the amyloid precursor protein gene and its regulation, and most recently, brain derived neuroinhibitors. The studies on the splicing of minigenes *in vitro* and *in vivo* systems offer the potential to look directly at gene regulators in the aging brain. Since alternately spliced forms of receptors show striking differences in function, it is possible that age changes occur. The association of the Cell Biology Unit contiguous to the Molecular Neurobiology Unit is leading to important collaborations. The further development of the Molecular Neurobiology Unit is a priority.

Age-Associated Deletions in Mitochondrial DNA: Several studies have shown that mutations and deletions in mtDNA are responsible for a variety of genetic disorders often involving myopathies or neurodegeneration. Also, it has been observed that the incidence of mtDNA with large deletions increases strikingly with age. Such deletions would cause a decline in energy production if they were present in a significant proportion of the mitochondria in a cell. We have carried out related studies with rats and found that their mtDNA show similar deletions with age. Thus, these deletions are not simply due to an accumulation of deficits with time but are related to aging per se. This model is being utilized to examine the cause(s) of the deletions (possibly oxygen radicals), the reasons for the striking increase in deletions with age, and possible ways to inhibit damage to mtDNA.



**Degeneration, Apoptosis, and Aging:** Changes are noted in a variety of cellular processes with age. Such changes include a reduced capacity for wound healing; loss of cartilage; increased incidence, but slower growth of tumors; reduced ability to heal fractures, etc. The possibility that apoptosis is increased is under investigation in several systems. Preliminary studies in Dr. Horton's laboratory have shown that chondrocytes undergo apoptosis when trophic factors are withdrawn or when they are treated with retinoic acid. The possibility that similar processes are involved in osteoarthritis is under study.

Other studies suggesting a role for apoptosis in aging come from studies using Matrigel, an extract of basement membrane proteins which has been used to study differentiation of endothelial cells in an *in vitro* angiogenesis assay. Preliminary experiments by Dr. Passaniti have shown that endothelial cell apoptosis occurs more rapidly when cells are growth-arrested, plated onto Matrigel, and trophic factors are withdrawn. Matrigel prepared from old animals appears to be especially potent in inducing apoptosis. These protein preparations may be a useful source of negative regulators (from old hosts) or trophic factors (from young hosts) which may influence vascularization and tissue regeneration and repair.

**Molecular Regulation of Chondrocyte Gene Expression:** With age and in osteoarthritis (OA), there are major changes in the proteoglycans of cartilage as well as alterations in the collagen genes expressed. Chondrocytes mediate many of the age-associated changes seen in cartilage and are central to the pathoetiology of OA. Studies in Dr. Horton's laboratory are directed toward identifying regulatory sequences in the gene coding for type II collagen and isolating chondrocyte factors that bind to these sequences. An understanding of these regulatory mechanisms could allow the development of strategies to stimulate cartilage repair and regeneration.

**Reversing Bone Loss:** Dr. Liang and his colleagues have found a number of significant differences in the response of old bone to trauma, in the number of stem cells, and in the response to growth factors. Drs. Liang and Williams have made the potentially important finding that minocycline, a tetracycline derivative which accumulates in bone, increases the density of the bones of old animals. Little or no effect was seen in adult animals, suggesting that an age-associated deficit was being reversed. Minocycline is used clinically to treat acne and is currently in a multicenter trial for the ability to inhibit the progression of rheumatoid arthritis. It is also under study for its ability to inhibit bone loss in periodontal disease. Minocycline, in addition to possessing antibiotic activity, has been shown by Golob and his collaborators to inhibit collagenase. We postulate that a reduction in bone resorption secondary to an inhibition of collagenase might explain the increase of bone density in old rats. Current studies are directed toward evaluating the quality of the bone it restores.

**Aging and Cancer:** Age, alone, is the single greatest risk factor for the development of cancer. While there are many possible explanations including a reduction in host surveillance, a longer exposure to carcinogens, the accumulation of mutations, etc. age-associated cancers adapted for growth in nude mice or cell culture have not been available. Dr. Passaniti has expanded the lines of human prostate cells available for study and developed improved methods to grow such tumors and to study their vascularization. Also, lines of cells have been developed from breast tumors arising spontaneously in aged female rats. These systems provide the opportunity to investigate the genetic events underlying the development of these cancers as well as explore various





methods of cancer prevention. Additionally, although the incidence of cancer increases with age, tumors such as prostate, breast, and ovarian, grow more slowly and progression is less aggressive in the elderly. Several mouse tumor models are being used to identify factors which may contribute to inhibition of tumor growth in aged animals. These factors are being tested in the human prostate tumor model in an effort to identify the source(s) of tumor growth inhibition with age.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00055-04 LBC

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

(Name, title, laboratory, and institute affiliation.)

Effect of Age on Osteogenic Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principle Investigator.)

C. Tony Liang, Research Chemist, LBC GRC NIA

Janices Barnes, Biologist, LBC GRC NIA

Sonia Williams, Staff Fellow, LBC GRC NIA

Quarto Rodolfo, Visiting Associate, LBC GRC NIA

Hiroshi Tanaka, Visiting Associate, LBC GRC NIA

COOPERATING UNITS (If any)

Dr. Mark Bolander, Orthopedic Surgery, Mayo Clinic

Dr. Russel Turner, Orthopedic Research, Mayo Clinic

Dr. Ron Smith, Southern Mississippi University

LAB/BRANCH:

Laboratory of Biological Chemistry

SECTION:

Regulatory Mechanisms Section

INSTITUTE AND LOCATION:

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS: 5.0 PROFESSIONAL: 4.0 OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The main goal of this study is to establish an animal model system which can be used to monitor age changes in bone activity which may relate to defects underlying osteoporosis and allow novel therapies to be tested. An injury model, involving bone marrow aspiration was used to induce rapid production of bone in the marrow cavity. This process can be quantitated by measuring the expression of genes involved in bone formation and resorption. The model was also used to examine the ability of aged animals to respond and indicated that bone induction was severely attenuated with age. Currently, we are using this model to evaluate the effectiveness of various novel interventions including growth factors, growth hormone and minocycline, an antibiotic with collagenase inhibitor activity, in restoring the ability of old animals to maintain bone mass.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
PROJECT NUMBER  
NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 AG 00056-03 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Mitochondria-Associated Tumor Hexokinase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

PI: C.R. Filburn Research Chemist, LBC GRC NIA

COOPERATING UNITS (if any)

P.L. Pedersen, Lab. Molecular Bioenergetics, Dept.Biol.Chem., Johns Hopkins Univ.

K.A. Arora, Lab. Molecular Bioenergetics, Dept.Biol.Chem., Johns Hopkins Univ.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cartilage Biology: Mechanisms & Models Related to Aging and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Walter E. Horton Jr., Senior Staff Fellow, LBC, GRC, NIA

Others:

Richard Balakir, Chemist, LBC, GRC, NIA

Patricia Precht, Biologist, LBC, GRC, NIA

Douglass Bradham, Staff Fellow, LBC, GRC, NIA

Liqun Wang, Visiting Fellow, LBC, GRC, NIA

Darryl Murray, Biologist, LBC, GRC, NIA

Georgeann Smale, IRTA, LBC, GRC, NIA

Imelda Udo, Junior Fellow, LBC, GRC, NIA

COOPERATING UNITS (If any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Cell Biology

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS: 4 PROFESSIONAL: 3 OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Cartilage is a unique tissue that functions to cushion the impact between bones and is a target for degenerative changes that result in osteoarthritis (OA), a disease afflicting millions of elderly individuals. OA involves changes in expression of matrix proteins such as collagen II as well as death of cells that comprise cartilage, the chondrocytes. We previously identified a region in the collagen II gene that functions as a chondrocyte-specific enhancer of transcription and further determined that a decamer sequence serves as binding site for chondrocyte-specific proteins. Recently, we demonstrated that a known DNA-binding protein (T-160, SSRP) binds to the enhancer region (although not to the decamer motif) and further that transcription factors belonging to the HLH family interact with the decamer sequence. Studies are ongoing to clone the chondrocyte-specific factors that bind to the enhancer. We are also studying the formation and degeneration of cartilage in various model systems. We have shown that factors associated with aged mice will suppress the formation of cartilage both *in vivo* and *in vitro*. Finally, we are testing the hypothesis that programmed cell death (apoptosis) may be responsible for the loss of chondrocytes that is observed with aging and OA.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PERIOD COVERED

October 1, 1992 to September 30, 1993

PROJECT TITLE (80 characters or less. Title must fit on one line between the borders.)

Aging, Angiogenesis, and the Growth and Spread of Tumors

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Antonino Passaniti, Senior Staff Fellow, LBC GRC

Joan Chang, Biologist Full-time, LBC GRC

Roberto Pili, Visiting Fellow, LBC GRC

Zohreh Naghashfar, Visiting Fellow, LBC GRC

Chunlin Yang, Visiting Fellow, LBC GRC

Melissa Zeman, SIS, LBC GRC

Cooperating Units (If any)

Hynda Kleinman, Chief, Cell Biology Section, LDB NIDR

Robert M. Taylor, Professor of Pathology, JHU FSK

Snorri S. Thorgeirsson, Chief, Lab. Exp. Carcinogenesis, NCI

Joseph di Paolo, Laboratory of Biology, NCI

Maurizio Capogrossi, Laboratory of Cardiovascular Sci., NIA

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Cell Biology

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

Total Man Years 5.25 Professional 4 Other 1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The incidence of cancer increases strikingly with age while the rate of tumor growth and spread is slowed. To explore the relation between cancer and aging we have adapted spontaneous human prostate and rat breast cancers occurring with age to transplantable lines to allow studies on their origins and properties. Further, we have developed a simple, quantitative assay to measure vascularization and assess angiogenic and potential anti-angiogenic factors. This system has been used to demonstrate that linomide, a useful anti-tumor drug, exerts its action by suppressing the vascularization of the tumor. We have also compared the growth of tumors in old and young hosts. These studies show a reduced rate of growth of a variety of tumor cells in old hosts. Extracts of tumor tissue grown in old hosts contain a factor(s) that reduces the growth of both normal and transformed cells and which could be a host factor not only slowing the growth of tumors but also impairing tissue repair and regeneration.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

Z01 AG 00059-03 LBC

PERIOD COVERED

October 1, 1992-September 30, 1993

TITLE OF PROJECT(80 characters or less. Title must fit on one line between the borders.)

Mitochondrial DNA Deletions: Role in aging and disease.

PRINCIPAL INVESTIGATOR

C.R. Filburn, Research Chemist, LBC GRC NIA

Others:

W. Edris, Biological Aide, LBC GRC NIA

M. Tamatani, Visiting Fellow, LBC GRC NIA

B. Young, Laboratory Aide, LBC GRC NIA

R. Cutler, Volunteer

COOPERATING UNITS (If any)

C. Stine, Assistant Professor, Johns Hopkins Univ., School Med.

R. Hansford, Senior Investigator, LCS, GRC, NIA

LAB/BRANCH

Laboratory of Biologicl Chemistry

SECTION

Regulatory Mechanism Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore., MD, 21224

TOTAL MAN-YEARS    2.5    Professional    2.0    Other    0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects    ☐ (b) Human tissues x    ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Damage to mitochondrial DNA (mtDNA) has been proposed to play a major role in the aging process. An increasing number of studies have shown that mtDNA mutations are involved in diseases involving muscle weakness and encephalopathy, but also include diabetes and other syndromes. In addition, marked increases in specific human tissues have been shown with increasing age. In an effort to assess the universality of this age-dependence, we have sought evidence of deletions in mtDNA in various tissues of young and old rats. We identified a 4.8 kb deletion which showed striking increases with age, perhaps suggesting common causes. The tissue distribution of the changes mimic those seen in human mitochondria with age. An altered form of mtDNA that appears to be an intermediate in the deletion process was also found and corresponds to one observed in human diseases. MtDNA damage leading to these changes may be important in muscle weakness, neurodegeneration and other age associated disabilities..



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation and Processing of Amyloid Precursor Protein Genes and Gene Products

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Jeffrey M. Chernak, Senior Staff Fellow, MNU LBC GRC NIA

## Others:

Peter W. Hoffman, IRTA Fellow, MNU LBC GRC NIA

Boyue Zhao, Visiting Fellow, MNU LBC GRC NIA

Michael Prenger, Biologist, MNU LBC GRC NIA

## COOPERATING UNITS (if any)

George Roth, Chief, MPGS LCMB GRC NIA

M. Maral Mouradian, Chief, GPU ETB NINDS

## LAB/BRANCH

Laboratory of Biological Chemistry

## SECTION

Molecular Neurobiology

## INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS: 4 PROFESSIONAL: 3 OTHER: 1

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major focus of our work is on the amyloid precursor protein (APP) and the role it plays in the neuropathology of Alzheimer's Disease (AD). We also initiated a study on the regulation of the D2 dopamine receptor gene and its role in Parkinson's Disease, tardive dyskinesia and normal aging. We made three independent mutations within the APP gene (mAPPs) which are known to cause early-onset, familial AD or Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch Type. Plasmids containing mAPPs were stably transfected into cell lines in order to examine phenotypic consequences to the cells and proteolytic processing of the expressed APPs. Neuronal and endothelial cells expressing mAPPs showed larger C-terminal fragments. NGF/cAMP treated PC-12 cells expressing mAPPs showed elevated levels of APP and C-terminal fragments. They had severely compacted cell bodies and eventually detached from the plates and died. We have cloned and characterized the 5' upstream regulatory region of the rat APP gene. A 375 bp fragment from this region functioned as a strong promoter in rat and human cell lines and its activity was enhanced by retinoic acid. The basal promoter activity was localized to a 75 bp sequence by deletion analysis. Finally, we isolated several DNA fragments from the functional promoter of the D2 dopamine receptor gene. Nuclear extracts from different tissues of young and old rats are being used with these promoter fragments to identify possible cis-acting transcriptional regulatory sites.



## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00501-02 LBC

## PERIOD COVERED

October 1, 1990 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alternative Splicing of the Amyloid Precursor Protein Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

PI: Gerald A. Higgins Research Biologist, MNU LBC GRC NIA

## Others:

Lisa Kale

Biologist

MNU LBC GRC NIA

Cheryl A. Sherman

Biologist

MNU LBC GRC NIA

## COOPERATING UNITS (if any)

Karen Beeman, Dept. of Biology, Johns Hopkins Univ.

## LAB/BRANCH

Laboratory of Biological Chemistry

## SECTION

Molecular Neurobiology Unit

## INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

2.0

1.0

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00503-02 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Neurotrophin Expression in Alzheimer's Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

PI: Gerald A. Higgins Research Biologist, MNU LBC GRC NIA

Others:

Carey Byrne

Biologist

MNU LBC GRC NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00504-02 LBC

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth Factor Activity in Neurodegeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

PI: Smita Kittur Senior Staff Fellow, MNU LBC GRC NIA

Others:

Hidetoshi Endo

Visiting Fellow

MNU LBC GRC NIA

COOPERATING UNITS (if any)

Dr. William Marksbery, M.D., Prof., Department of Neurology, University of Kentucky

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Unit

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

2.0

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Excitatory Amino Acid Receptors in Alzheimer's Disease & Neurodegenerative Disorders

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

John W. Kusiak, Research Chemist, MNU LBC GRC NIA

Others:

Darrell D. Norton, Chemist, MNU LBC GRC NIA

John A. Izzo, IRTA (April 1992), MNU LBC GRC NIA

Cheryl A. Sherman, Biologist (June 1992), MNU LBC GRC NIA

Guang Bai, Sr. Staff Fellow (July 1992), MNU LBC GRC NIA

COOPERATING UNITS (if any)

Molecular Physiology & Genetics Section, LCMB, GRC, NIA

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS: 5

PROFESSIONAL: 3

OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The major emphasis of this project is the discovery of the role of EAA receptors in neurodegenerative disorders of aging. This year we identified additional isoforms of the NMDAR1 receptor subunit and we measured changes in receptor mRNA isoform expression by RNase protection. We detected an approximate 20% increase in hippocampus in an isoform lacking several phosphorylation sites in the carboxyl terminal in aged rats. Mini-gene constructs of the GLUR-2 glutamate/AMPA receptor subunit were constructed and transfected into cultured cells in order to examine the mutually exclusive splicing of two exons important in regulating agonist sensitivity of this subunit. We discovered that eliminating a downstream branch point led to exon exclusion and surprisingly, use of an upstream cryptic donor site. We cloned and characterized the promoter region of the NMDAR1 receptor. We identified two major transcription start sites at nucleotides -276 and -238 upstream of codon 1. Proximal to the transcriptional start site are one GSG and two SP1 regulatory motifs but no TATA or CAAT boxes. These results suggest that the NMDAR1 receptor gene has characteristics of a housekeeping gene but may be regulated by immediate-early genes. Reporter gene constructs of this promoter region show strong positive and strong negative elements. We are currently studying the role of NGF in regulating NMDAR1 gene expression.



LCS

LCMB

ICP

IMG

IPC





ANNUAL REPORT OF THE LABORATORY OF CARDIOVASCULAR SCIENCE  
NATIONAL INSTITUTE ON AGING

The overall goals of the Laboratory of Cardiovascular Science are (1) to identify age-related changes that occur within the cardiovascular system and to determine the mechanisms for these changes; (2) to study myocardial structure and function and to determine how age interacts with these chronically altered cardiac states to determine the level of myocardial function; (3) to study basic mechanisms in excitation-contraction coupling and of energy-yielding oxidative pathways in cardiac muscle; (4) to determine the chemical nature and sequence of intermediate reactions controlling the movement of ions through ionic channels and pumps present in myocardium, specifically with respect to how the affinity, capacity and selectivity of ion translocation through membranes are affected by aging and disease; and (5) to determine mechanisms that govern normal and abnormal function of vascular endothelial cells. In meeting these objectives, studies are performed in human volunteers, intact animals, and isolated heart and vascular tissues, isolated cardiac and vascular cells, and subcellular organelles. Our studies during FY93 are summarized below.

CARDIAC STUDIES IN HUMANS

Effects of Age, Gender and  $\beta$  Blockade on Resting and Exercise Cardiac Performance.

A. Although the effects of age on the cardiac response to maximal aerobic exercise are well known in men, whether women undergo similar aging changes is unknown. To determine the independent effects of age and gender on the left ventricular response to exercise, we performed gating blood pool scans at rest and maximal upright cycle exercise in 121 men and 79 women ages 22-86 yrs free of heart disease by history, physical exam, rest and exercise ECG and if > 40 yr old, exercise thallium scan. Maximal cycle workload declined similarly with age in men (36%) and women (42%) between the third and ninth decades, although the absolute maximal load achieved was higher in men for any given age. At seated rest age-associated declines in heart rate (HR) and increases in systolic blood pressure (SBP) were observed in both sexes. Resting end-diastolic volume index (EDVI) and stroke volume index (SVI) rose with age in men but not in women. In both sexes, maximal heart rate, ejection fraction and cardiac index declined with age whereas end systolic volume index (ESVI) and total systemic vascular resistance (TSVR) increased. Although EDVI at maximal effort increased with age in men but not in women, SVI was not age related in either sex. Thus, aging and gender have distinct influences on the cardiac response to maximal cycle exercise. B. The response to strenuous aerobic exercise (EX) is mediated in large part by beta-adrenergic activation, the efficiency of which declines with advancing age. To ascertain the importance of the beta-adrenergic system on age-associated changes in hemodynamic during EX, we performed maximal cycle EX in 25 healthy men ages 28-72 yr from the BLSA after acute beta blockade with intravenous propranolol. In these men, EDVI at peak workload declined with age ( $r = -0.45$ ) causing an age-associated decline in SVI ( $r = 0.48$ ,  $p < 0.05$ ) not present in 70 unblocked men. The decline in HR with age in propranolol-treated men was blunted ( $0.46$  beats/min/yr) compared to controls ( $1.09$  beats/min/yr). Maximal LVEF declined with age similarly with ( $r = -0.50$ ,  $p < .01$ ) and without ( $r = -0.45$ ,  $p < .0001$ ) beta-adrenergic blockade. The primary reason for the slope shifts in the age regressions propranolol was a large increase in EDVI and SVI and a large decrease in HR in younger men. Similarly, IV propranolol resulted in reduced peak filling rates with EX in young ( $27 \pm 8$  yr) but not older ( $62 \pm 6$  yr) men relative to their unmedicated age peers. We conclude that age differences in beta-adrenergic responsiveness underlie many of the age-associated changes in hemodynamic during vigorous aerobic EX.



## Effect of Physical Conditioning on Cardiovascular Aging Changes in Normal Man

A. It is not known whether central or peripheral adaptations are primarily responsible for the markedly increased aerobic performance of endurance trained older men relative to their sedentary age peers. To answer this question, we performed maximal upright cycle ergometry in 16 endurance trained men  $63 \pm 7$  yr old and 35 untrained men of similar age from the BLSA. During cycle ergometry, trained men achieved higher maximal workloads, WL, ( $177 \pm 28$  vs  $131 \pm 28$  watts,  $p < .0001$ ) and higher peak  $\dot{V}O_2$  ( $34.2 \pm 3.6$  vs  $22.3 \pm 5.8$  ml/kg/min,  $0 < .0001$ ). The higher peak  $\dot{V}O_2$  in trained men was achieved by a 22.5% higher cardiac index, CI, ( $9.8 \pm 1.9$  vs  $8.0 \pm 1.5$  l/min/m<sup>2</sup>,  $p < .001$ ) and a 16.5% greater arteriovenous  $O_2$  difference ( $13.3 \pm 2.6$  vs  $11.5 \pm 3.0$  vol/100ml,  $p < .05$ ). The higher maximal CI in the athletes was mediated entirely by a higher stroke volume index, SVI, ( $68.7 \pm 10.5$  vs  $57.5 \pm 11.3$  ml/kg/m<sup>2</sup>,  $p < .002$ ). Thus, the augmented aerobic capacity of endurance trained older men during upright cycle exercise (EX) is achieved by both central and peripheral adaptations, which are of similar magnitude. B. It has been hypothesized that age-associated reductions in physical conditioning status mediates the decrease in LV early diastolic performance seen with advancing age. To test this hypothesis, we measured radionuclide ventriculographic peak filling (PF) rates at rest and throughout graded maximal upright cycle ergometry in 56 sedentary BLSA men and 12 highly trained old men. At rest, at 50% of maximum WL and at maximal effort, PF rates declined with age but were similar in older athletes and their sedentary age peers. These results suggest that the age-associated decline in early diastolic filling (DF) observed at rest and during EX cannot be reversed even by intensive long-term endurance training. C. To determine the effect of deconditioning on LV DF in endurance trained older men, 9 such men underwent serial radionuclide ventriculography at rest and during maximal upright cycle EX before and after 12 weeks of deconditioning. Although resting PF rates and cardiac volumes did not change significantly after deconditioning, at peak EX WL, PF rate ( $893 \pm 197$  vs  $1124 \pm 319$  ml/sec,  $p < .05$ ) as well as end-diastolic volume index ( $73 \pm 13$  vs  $84 \pm 17$ ,  $p < .05$ ) and SVI ( $59 \pm 11$  vs  $71 \pm 14$  ml/m<sup>2</sup>,  $p < .05$ ) were reduced after deconditioning. These findings suggest that the decrease in LV volumes at peak EX seen after deconditioning in highly trained seniors may be mediated by a reduction in diastolic early PF rate.

## Age-Associated Changes in Cardiac Rhythm and Conduction

A. In subjects without known organic heart disease, ventricular arrhythmias precipitated by exercise or occurring during routine activity increase in frequency and complexity with age and cause substantial cardiac morbidity. Although increased echocardiographic left ventricular (LV) mass predicts a higher prevalence and complexity of ventricular arrhythmias (VA) on ambulatory ECG, whether such a relationship between LV anatomy and exercise-induced VA (EIVA) is unknown. We therefore examined this question in 288 healthy BLSA volunteers 20-90 years old who underwent both M-mode echocardiography and maximal treadmill exercise testing. Simple, i.e. isolated, EIVA occurred in 53 subjects (18%) and complex EIVA (comparing  $\geq 10\%$  of beats in any minute or occurring in runs) in 15 subjects (5%). Although univariate predictors of any EIVA were greater LV mass ( $p = .0006$ ), older age ( $p = .0009$ ), larger body surface area ( $p = .003$ ), higher peak systolic blood pressure ( $p = .01$ ) and male sex ( $p = .01$ ), only age ( $p = .002$ ) independently predicted EIVA by multiple logistic regression analysis. B. Although respiratory sinus arrhythmias (RSA) is known to decrease with advancing age, the independent effects of age, gender, conditioning status and body composition are unknown. The impact of age, fitness, gender and relative weight on resting heart rate variability was examined in 117 healthy normotensive adults ages 19-82 from the BLSA; heart rate variability was indexed by RSA, extracted from a 3-minute seated ECG using time domain digital filtering. By linear regression analysis, RSA varied inversely with age ( $r = -0.61$ ,  $p < .001$ ) and body mass index ( $r = -0.31$ ,  $p < .01$ ), directly with  $\dot{V}O_{2max}$  ( $r = 0.40$ ,  $p < .001$ ) and was unrelated to gender. Multiple regression analysis demonstrated that age and body mass but not  $\dot{V}O_{2max}$  were independent predictors of RSA. C. To examine the hypothesis that heart rate variability (HRV) is reduced in apparently healthy



subjects with latent coronary artery disease (CAD), we measured resting ECG R-R interval variations in 29 asymptomatic male volunteers aged  $61.8 \pm 11.2$  years, free of clinical heart disease, who developed a coronary event (CE), within the next 2 years. Compared to 58 age-matched controls (C) who remained event free for  $12.7 \pm 6.5$  years after the index ECG, men destined for a CE showed smaller standard deviation of R-R ( $26.1 \pm 10.8$  vs  $37.9 \pm 24.0$  msec,  $p = .002$ ) and smaller absolute difference between minimum and maximum R-R ( $98.6 \pm 40.3$  vs  $136.7 \pm 81.0$  msec,  $p = .004$ ) indicating reduced HRV in men with latent CAD.

## MECHANISTIC STUDIES OF INTRINSIC CARDIAC MYOCYTE FUNCTION

### Excitation-Contraction Mechanisms in Isolated Cardiac Cells

The coupling of surface membrane depolarization of heart cells to contraction is mediated by a release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR). The precise mechanism of coupling of the depolarization to SR  $\text{Ca}^{2+}$  release is presently the preeminent issue in cardiac biophysics. Morphological studies of cardiac cells have shown that the junctional SR, from which  $\text{Ca}^{2+}$  is released upon stimulation, forms structural "couplings" with the sarcolemma (SL), primarily (by 80%) with the T tubules, i.e. invaginations of the SL into the cell interior. At the "couplings", the SL  $\text{Ca}^{2+}$  channels and the SR  $\text{Ca}^{2+}$  release channels span the gap between closely apposed but separate SL and SR membrane systems. It has been generally postulated, therefore, that a direct regulatory effect of the L-type  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) on the SR  $\text{Ca}^{2+}$  release, typical for mammalian ventricular myocytes, results from a preferential access of the  $\text{Ca}^{2+}$  current to the SR  $\text{Ca}^{2+}$  release sites via the "couplings". The present project is aimed on testing this hypothesis by comparing the dependence of SR  $\text{Ca}^{2+}$  release on the L-type  $\text{Ca}^{2+}$  current in mammalian (rat) ventricular myocytes, which have an extensive T tubular system and extensive "couplings", and avian (Finch) ventricular myocytes, which lack the T tubules and, therefore, over 80% of its junctional SR lacks contact with the SL. Rat and Finch ventricular cells are isolated from the heart (collagenase digestion), loaded with a  $\text{Ca}^{2+}$  indicator (indo-1/FA) via a patch pipette, and voltage clamped (whole cell patch method). Test depolarizations lasting 25 to 250 msec to potentials that span the range of the L-type  $\text{Ca}^{2+}$  channel activation are then made and  $I_{\text{Ca}}$  and  $\text{Ca}^{2+}$  transient amplitude are measured. The results obtained so far show that in bird ventricular myocytes the SR  $\text{Ca}^{2+}$  release is well correlated to  $\text{Ca}^{2+}$  influx via the L-type  $\text{Ca}^{2+}$  channels and is qualitatively similar to the relationship between  $I_{\text{Ca}}$  and the SR  $\text{Ca}^{2+}$  release observed in rat ventricular myocytes. Thus, the results suggest that the direct control of SR  $\text{Ca}^{2+}$  release by  $I_{\text{Ca}}$  is not attributable to a preferential access of  $\text{Ca}^{2+}$  influx to SR  $\text{Ca}^{2+}$  release sites via the SL-SR "couplings". This apparently necessitates a change of the current dogma regarding the functional significance of these "couplings". Specifically, models of excitation-contraction coupling in which  $\text{Ca}^{2+}$  diffusion is more extensive perhaps in series with local control models (20% of coupling in Finch) will need to be explored.

### Role of $\text{Ca}^{2+}$ /Calmodulin-Dependent Protein Kinase II in Heart $\text{Ca}^{2+}$ Channel Regulation

$\text{Ca}^{2+}$  current flowing via sarcolemmal  $\text{Ca}^{2+}$  channels of heart cells is an important determinant of cell  $\text{Ca}^{2+}$  homeostasis and thus an important determinant of a variety of cell functions. While  $\text{Ca}^{2+}$  and membrane voltage-dependent positive regulation of  $\text{Ca}^{2+}$  channels have been recently reported, little is known of the mechanism. Our studies provide compelling evidence that in adult rat cardiac ventricular cells  $\text{Ca}^{2+}$ /calmodulin dependent kinase II mediates L-type  $\text{Ca}^{2+}$  current facilitation, i.e., an augmentation in current magnitude and/or slowing of its inactivation during repetitive depolarizations, by single strong depolarizing prepulse or by depolarizing holding potentials. All of these effects on the current were completely abolished by a inclusion of specific peptide inhibitor of  $\text{Ca}^{2+}$ /calmodulin kinase II within the pipette filling solution or by the replacement of  $\text{Ca}^{2+}$  with  $\text{Ba}^{2+}$  in bath solution. The involvement of  $\text{Ca}^{2+}$ /calmodulin kinase II on  $\text{Ca}^{2+}$  current regulation is further supported by the



localized distribution of a specific antibody to the kinase near cell sarcolemma as revealed by digital confocal fluorescent imaging. Furthermore, the immunofluorescence of the antibody was significantly enhanced by depolarization with high  $[K^+]_o$  and attenuated by removal of  $Ca^{2+}_o$  or by W7, a calmodulin inhibitor. Thus,  $Ca^{2+}$ /calmodulin kinase II dependent protein phosphorylation is an important mechanism by which variable factors, such as  $Ca^{2+}$ , repetitive stimulation and strong depolarization can positively regulate  $Ca^{2+}$  current in heart cells, and that membrane potential plays an essential role in the modulation of the kinase activity. These findings provide new insights toward understanding  $Ca^{2+}$  channel regulation in cardiac cells as possibly in other cell types as well.

#### Secondary $[Ca^{2+}]_i$ -Dependent Modulation of Contractility in Single Cardiac Myocytes

It is well established that the primary modes of altering myocardial contractility on a beat-to-beat basis involve both the modulation of the myoplasmic  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) availability at the level of the myofilaments (MF), and changes in the  $Ca^{2+}$ -response of the MF, especially via length-dependent activation. Alternatively, certain more slowly developing covalent modifications of MF have been recognized (such as thick or thin filament phosphorylation), but these are of uncertain functional significance in cardiac tissue. We have found that the  $[Ca^{2+}]_i$  "history" regulates contractility via myosin light chain (MLC<sub>2</sub>) phosphorylation, based on the results of  $[Ca^{2+}]_i$ -clamp experiments at various levels (ranging up to 10-fold > resting  $[Ca^{2+}]_i$ ) and durations (up to 60s) via 10 Hz tetanization in sarcoplasmic reticulum-disabled (thapsigargin or caffeine treated) intact rat cardiac myocytes loaded with indo-1 free acid. The rising phase of the tetanus (2-3 sec) was sufficiently slow to permit continuous equilibration of  $Ca^{2+}$ -MF binding, and thus force and cell length, enabling a rapid assessment of baseline contractile activation. Secondary  $[Ca^{2+}]_i$ - and time-dependent progressive cell shortening was observed despite steady levels of clamped  $[Ca^{2+}]_i$ , representing a progressive leftward shift in the length-pCa curve (without changing  $F_{max}$  nor the Hill coefficient) due to MLC<sub>2</sub> phosphorylation. The effect of protein phosphatase inhibitor calyculin A, which (nonspecifically) increases MLC<sub>2</sub> phosphorylation and left-shifts the L-pCa curve (without changing  $F_{max}$ ) was rapidly reversed by the nonspecific phosphatase 2,3-butanedione monoxide (BDM), which reduces MLC<sub>2</sub> phosphorylation and  $F_{max}$ , and shifts the L-pCa curve to the right. We propose that  $[Ca^{2+}]_i$  history, via  $Ca^{2+}$ -calmodulin-dependent MLCK activity, myosin light chain phosphorylation, and kinase/phosphatase balance, plays a significant modulatory role in the chronic regulation of contractility in intact heart cells. This integrative phenomenon would reflect a history dependence of cardiac work/demand, and may serve as an important cardiovascular adaptive mechanism in myocardial conditioning.

#### Fatty Acid Modulation of L-Type $Ca^{2+}$ Channel Function in Cardiac Myocytes

Antiarrhythmic effects of polyunsaturated fatty acids following dietary incorporation into cardiac cell membranes have been observed in recent years. The mechanisms of action are yet to be defined. In this study the effect of membrane-free polyunsaturated fatty acids delivered to isolated adult rat cardiac myocytes was investigated (in vivo these can be released from cardiac cell membranes following phospholipase action). The present study investigates the effects of DHA and arachidonic acid (AA; C20:4, n-6) on L-type calcium channel conductance in whole cell voltage-clamp experiments and on cytosolic free calcium fluorescence and contraction in adult rat indo-1 loaded cardiac myocytes. The effect of DHA on dihydropyridine interaction with  $Ca^{2+}$  channel current ( $I_{Ca}$ ) and twitch contraction in isolated myocytes (25°C) was measured. Nitrendipine (10 nM) reduced peak  $I_{Ca}$ , measured by whole cell clamp from -40 to -5 mV, twitch contraction amplitude and associated cytosolic indo-1  $Ca^{2+}$  fluorescence measured during electrical stimulation (0.5Hz). DHA (5  $\mu$ M) abolished these effects but AA did not block the nitrendipine effects. Experiments with 10nM BAYK8644 resulted in increased twitch contraction and related cytosolic calcium which could be prevented by DHA but not AA (Fig2). DHA or AA alone had no effect. Neither DHA





nor AA altered isoproterenol (1, 0.5, 0.1  $\mu$ M) induced increases in  $I_{Ca}$  or twitch amplitude. That DHA abolishes nitrendipine or BAYK8644 effects but has no effect alone, suggests that it binds to  $Ca^{2+}$  channels near dihydropyridine binding sites and interferes with  $I_{Ca}$  modulation by dihydropyridines. This action may be involved in the antiarrhythmic effects of fish oil diet in vivo both in animal models and in humans with coronary artery disease.

#### Dietary Fatty Acid Modulation of Myocardial Function and Influences on Aging

The period of cardiac myocyte contraction, the transmembrane action potential and cytosolic  $Ca^{++}$  transient are extended in old rat cardiac cells compared to those from young adults. The rate of sarcoplasmic reticulum uptake of  $Ca^{++}$  and the level of  $Ca^{++}$  stimulate ATPase activity decreases with senescence and the cardiac response to  $\beta$ -adrenergic stimulation is reduced with aging. It is proposed that "normal" age-related changes in cardiac function and increased cardiac pathology with age may be associated with alterations to membrane composition which may be intervened by dietary lipid modification of myocardial cell and intracellular membranes. The vulnerability to arrhythmic stimuli increases with age. Short term feeding with n-3 polyunsaturated fatty acid (n-3 PUFA) rich diet in old rats and monkeys significantly reduces the incidence of ventricular arrhythmias whereas saturated fat rich diet (SAT) is pro-arrhythmogenic. SAT diet induces a marked increase in myocardial  $O_2$  demand independent of contractile function under control conditions. In contrast  $O_2$  demand was very low following n-3 PUFA diet. Coronary vasodilator reserve was greater following n-3 PUFA diet than SAT diet. These differences are not due to any change in basal metabolism or vascular function but rather to intracellular  $Ca^{++}$  homeostasis. The present study (commenced January 1993), investigates the effect of dietary lipid modulation in isolated cardiac myocyte membranes in order to test whether total intracellular  $Ca^{++}$  or  $Ca^{++}$  redistribution between the cytosol and organelles is altered. Cytosolic free calcium fluorescence (indo-1) and twitch amplitude of electrically stimulated isolated cardiac myocytes are currently being measured from n-3 PUFA or saturated fat dietary treated rats (6,12,24 mo). The responsiveness to the stresses of high and low stimulation rates (0.5, 2Hz), BAYK8644 (L-type  $Ca^{++}$  channel agonist), isoproterenol ( $\beta$ -adrenergic receptor agonist) and hypoxia is also to be assessed in these groups. Cell membrane fatty acid profile analysis is currently in progress to confirm the extent of change to cardiac membrane lipid composition.

#### Ion Transport Mechanisms

In eukaryotic cells, transmembrane gradients for  $Na^+$ ,  $K^+$  and  $Ca^{2+}$  are established and maintained by ATP-dependent ion pumps. Deterioration of these systems with age can lead to alterations in cellular ion homeostasis and loss of cell function. This study focuses on the basic transport mechanisms utilized by ion pumps to gain insight into the molecular basis of altered transport function during aging.

The biochemical expression of the plasma membrane Na,K pump is an ATPase which cycles through phosphorylated and dephosphorylated intermediate states. The kinetic behavior of the ATPase partial reactions is complex and cannot be explained by a simple consecutive mechanism. Recent rapid mixing experiments have shown that some of these complex effects can be eliminated by treatment with non-solubilizing concentrations of n-dodecyl  $\beta$ , D maltoside, a non-ionic detergent. We propose that interactions between  $\alpha$  catalytic subunits are responsible for the complex behavior and that the detergent disrupts hydrophobic subunit-subunit contacts which are necessary for optimal pumping rates and energy utilization.



Rapid mixing and time-resolved EPR studies of the  $\text{Ca}^{2+}$  pump in sarcoplasmic reticulum have produced evidence for a substrate-activated conformational state which participates in  $\text{Ca}^{2+}$  translocation. Relaxation of this conformational state is associated with a large free energy change resulting in  $\text{Ca}^{2+}$  release into an occluded compartment near the inner membrane surface. The kinetic behavior of the Ca-ATPase is compatible with a dimer in which energy-yielding and energy-requiring reactions in adjacent subunits are coupled to optimize  $\text{Ca}^{2+}$  pumping.

## REGULATION OF ENERGY METABOLISM IN AGING AND DISEASE

### Cellular and Subcellular Calcium Ion Homeostasis

This project focuses on the mechanism whereby cells achieve homeostasis of  $\text{Ca}^{2+}$  ion concentration, both within the cytosol and other cellular compartments, and allow changes in  $\text{Ca}^{2+}$  in response of hormones and neurotransmitters. We are currently focussing on the role of deranged  $\text{Ca}^{2+}$  homeostasis in the process of cell death. This year we have investigated the effect of the overexpression of the bcl2 oncogene in the Jurkat T cell line upon regulation of cytosol  $[\text{Ca}^{2+}]$  and the thapsigargin-sensitive pool of endoplasmic reticulum  $\text{Ca}^{2+}$ . In response to serum-starvation, the bcl2-transfected cells survive longer and maintain a larger thapsigargin-releasable pool of  $\text{Ca}^{2+}$  than do the control cells. There may be a cause-and-effect relationship between these two parameters.

We have also asked the question of whether killing of cells by exposure to visible light in the presence of porphyrins involves the peripheral benzodiazepine receptor in the mitochondrial membrane, the opening of the mitochondrial "megachannel" and the consequent release of a pulse of  $\text{Ca}^{2+}$  into the cytosol. In studies with mitochondrial isolated from heart and liver, exposure to porphyrins and light was found to be extremely effective in inhibiting mitochondrial  $\text{Ca}^{2+}$  uptake and in causing release of accumulated  $\text{Ca}^{2+}$ . However, the effect was not blocked by cyclosporin, which inhibits the opening of the megachannel, and did involve uncoupling, as shown by fluorescence of the membrane potential-sensitive dye JC-1. Thus, the induction of damage by photodynamic therapy is broader in mechanism than the opening of this channel alone. These studies on  $\text{Ca}^{2+}$  in cell death are geared towards an understanding of the basic biology of this process, with an eventual goal of designing interventions to prevent unwanted cell death in fixed, post-mitotic tissues during aging.

### Regulation of Energy Metabolism in Aging and Disease: Cardiovascular System

This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise tissue survival. (1) We have focused this year on the relation between altered mitochondrial  $\text{Ca}^{2+}$  ion homeostasis and the potential for oxidative phosphorylation. We have extended our previous work on cardiac myocytes from the cardiomyopathic Syrian hamster. Studied at the point of failure, these hearts perform less work and also fail to activate pyruvate dehydrogenase as completely as in healthy hearts. In the previous year we tied this to a failure to elevate intramitochondrial free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_m$ ) in cells from myopathic animals, when  $[\text{Ca}^{2+}]_m$  was studied in individual cardiac myocytes subjected to an electrical pacing regimen. This year we showed that the likely cause of the diminished response of  $[\text{Ca}^{2+}]_m$  to increased frequency of electrical stimulation in the cardiomyopathic is the generation of smaller systolic transients in cytosolic



free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) in myocytes from the failing hearts. (2) We have extended the previous year's studies on the dependence of  $[\text{Ca}^{2+}]_i$  upon frequency of electrical stimulation, to separate an effect due to  $\beta$ -adrenergic stimulation from that of frequency. Further, we have used the effect of mitochondrial uncoupling agents to provide an independent verification of our finding that the mitochondrial  $\text{Ca}^{2+}$  gradient may be positive or negative, depending upon the degree of cell stimulation.

#### Regulation of Energy Metabolism in Aging and Disease: Central Nervous System

This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise survival of neurones. We have investigated the frequency of occurrence of a major deletion (4.8 Kb) in mitochondrial (mt) DNA in four defined brain regions (cerebral cortex, cerebellum, hippocampus and striatum), as a function of aging in the rat. mt-DNA codes for subunits of complexes I, III and IV of the mitochondrial respiratory chain as well as complex V (the ATP-synthase), ribosomal and t-RNA's. The magnitude of the major deletion which we have studied is such as to make transcription completely incompetent for the affected DNA molecule; however, complementation with other DNA molecules within the same mitochondrion is likely to occur. We found increases with aging of approximately 6, 10 and 20-fold in the incidence of the 4.8 Kb deletion, as a fraction of total genomes, when cerebral cortex, hippocampus and striatum, respectively, were compared in 6 month and 22-23 month old rats. However, the deleted genomes still represent a very small fraction of the total (less than 1%) in old-age. Whether they are important in the functioning of the tissue likely depends upon (a) whether distribution is even, or if instead there is mosaicism among neurons, and (b) the complement of other forms of mutation which also prevent transcription of mt-DNA. This is a joint project with Dr. C.F. Filburn of LBC, NIA.

#### MECHANISMS OF RECEPTOR MEDIATED ACUTE MODULATION OF CARDIAC CELL PERFORMANCE

##### Comparison $\beta_1$ vs $\beta_2$ Adrenoceptor Stimulation in Rat Cardiocytes

$\beta$ -adrenergic receptor ( $\beta$ AR) stimulation has profound modulatory effects on the cardiac contraction. It has been well documented that both the  $\beta_1$  and  $\beta_2$  AR subtypes are coupled to adenylcyclase and that their stimulation by  $\beta_1$  and  $\beta_2$  AR specific agonist leads to an increase in cAMP. Stimulation of other heart cell receptors, e.g. prostaglandin, also leads to an increase in cAMP but has no effect on contraction, presumably because the cAMP pool affected is not associated with membranous cAMP activation and is not linked to heart cell  $\text{Ca}^{2+}$  regulation. It is widely recognized that stimulation of  $\beta$  AR's leads to an increase in the particulate (membrane bound) cAMP levels and protein kinase (PK) phosphorylation of key proteins involved in excitation-contraction coupling. However, whether  $\beta_2$  AR stimulation increases particulate cAMP and cAMP dependent phosphorylation is not known. In this regard we have recently shown that the  $\beta_2$  AR mediated effects on  $\text{Ca}^{2+}$ , contraction and L type  $\text{Ca}^{2+}$  channels in rat heart cells differ markedly from those elicited by  $\beta_1$  AR stimulation. In this project we further demonstrated that the  $\beta_2$  effects on  $\text{Ca}^{2+}$  and contraction in these cells are not mediated by cAMP. This conclusion is based on the measurement of total and particulate cAMP levels and the decoupling between the increase of cAMP levels and increases in cell contraction and  $\text{Ca}_i$  transient. Furthermore, phosphorylation of sarcoplasmic reticulum (SR) phospholamban was dramatically increased by  $\beta_1$  AR stimulation, but not by  $\beta_2$  AR stimulation. Thus,  $\beta_1$  and  $\beta_2$  AR are



coupled to cellular effects of altered  $\text{Ca}^{2+}$  homeostasis and contraction via different signal transduction pathways.

#### $\alpha$ -Adrenergic Stimulation in Myocardial Cells

We have previously shown that the positive inotropic action of  $\alpha_1$ -adrenoceptor ( $\alpha_1$ -AR) stimulation, at least in part is due to an enhanced myofilament responsiveness to  $\text{Ca}^{2+}$  mediated by protein kinase C (PKC)-dependent activation of  $\text{Na}^+/\text{H}^+$  exchange and an increase in cytosolic pH ( $\text{pH}_i$ ). We have also examined the effect of  $\alpha_1$ -AR subtypes,  $\alpha_{1A}$  and  $\alpha_{1B}$  on contraction,  $\text{Ca}_i$  and myofilament response to  $\text{Ca}^{2+}$  of isolated myocardial cells.  $\alpha_{1A}$ -AR stimulation (phenylephrine, nadolol and  $\alpha_{1B}$ -AR inactivation with chloroethylclonidine) increased contraction,  $\text{Ca}_i$  transient amplitude and myofilament response to  $\text{Ca}^{2+}$ . In contrast  $\alpha_{1B}$ -AR stimulation (phenylephrine, nadolol and  $\alpha_{1A}$ -blockade with WB-4101) decreased contraction,  $\text{Ca}_i$  transient amplitude and downregulated the  $\alpha_{1A}$ -AR effect to increase myofilament response to  $\text{Ca}^{2+}$ . In additional experiments we have examined the effect of  $\alpha_{1A}$ - and  $\alpha_{1B}$ -AR stimulation on  $\text{pH}_i$  and the PKC-dependence of the  $\alpha_{1B}$ -AR effect on  $\text{pH}_i$  and contraction of isolated myocardial cells. In  $\text{HCO}_3^-/\text{CO}_2$ -buffered saline,  $\alpha_{1A}$  increased cytosolic pH. In contrast  $\alpha_{1B}$  decreased cytosolic pH and this effect persisted in  $\text{HCO}_3^-/\text{CO}_2$ -free solution.  $\alpha_{1B}$ -mediated cytosolic acidification was abolished by staurosporine, a protein kinase C inhibitor, and by protein kinase C down-regulation with prolonged exposure to 4 $\beta$ -phorbol 12-myristate 13-acetate. Changes in twitch amplitude paralleled those in cytosolic pH due either to  $\alpha_{1A}$ - or  $\alpha_{1B}$ - adrenoceptor stimulation. Our results show that  $\alpha_{1A}$ -AR and  $\alpha_{1B}$ -AR have opposite effects on  $\text{pH}_i$  homeostasis of isolated myocardial cells.

#### **CARDIAC GENE EXPRESSION**

##### Age-Associated Patterns of Gene Expression in the Heart

Atrial natriuretic factor (ANF) is synthesized and secreted by atrial tissue in response to experimental or genetic hypertension. Recent studies have shown that ANF is produced and secreted by left ventricular tissue as well, and that ANF has direct effects on ventricular cardiac myocyte contraction. Since heart contractile function is altered during aging, and the senescent heart exhibits many other phenotypic and genotypic characteristics of the hypertensive heart, we tested the hypothesis that the atrial natriuretic factor (ANF) gene would be upregulated with advancing age. Since progressive age-associated myocyte hypertrophy is evident in left ventricle (LV) of the Wistar, but not in the LV of F344 nor in the right ventricle (RV) of either strain, total RNA was isolated from the LV and RV of male Wistar and Fischer<sup>†</sup> rats aged 1.5-27 mo of age. Northern blots were probed with a radiolabeled cDNA probe synthesized by PCR using oligonucleotides complementary to the published sequence. The levels of mRNA coding for ANF increased progressively with advancing age in ventricles of both strains of rats. ANF mRNA abundance was 7-fold greater in ventricles of senescent compared to young adult rats. In freshly isolated ventricular myocytes, a similar pattern was observed. ANF mRNA levels were not augmented during aging in the atria of Wistar rats. In contrast to the age-associated increase in ventricular ANF mRNA levels, the concentration of ANF peptide in the LV decreased with advancing age, suggesting that either secretion or degradation rates are increased in the ventricles during aging. To obtain an indication of hemodynamic stress, expression of early response genes was assessed. Levels of both heat shock protein (hsp70) and c-jun mRNAs were elevated  $\approx$ 2-fold in hearts





of some, but not all senescent rats, compared to those of young adult rats. c-fos and junB mRNAs were not elevated in any of the hearts studied. The low level induction of hsp70 and c-jun gene expression in some senescent hearts, suggests that a subpopulation of myocytes and/or fibroblasts may be stressed in the aging heart. Since in F344 LV and in the RV of both strains the age-associated elevation in ANF occurs in absence of myocyte hypertrophy, these results suggest that an independent age-related mechanism exists to regulate ANF gene expression in the ventricles. Further study is required to identify factors regulating ANF gene expression in the ventricles during aging, and to determine the fate of ANF peptide in the senescent heart.

#### Cardiac Gene Expression During the Transition from Hypertrophy to Heart Failure

Heart failure is a progressive disease with extremely poor prognosis. The failing heart is characterized by impaired cardiac muscle function and increased interstitial fibrosis. The mechanisms initiating the transition from stable hypertrophy to irreversible heart failure are unknown. Our purpose was to determine whether the functional impairment of the failing heart is associated with changes in levels of mRNA encoding proteins responsible contraction and relaxation, and whether the increased fibrosis in the failing heart is related to induction of genes encoding extracellular matrix components. We studied hearts of 18-24 mo spontaneously hypertensive rats with signs heart failure (SHR-F) or without evidence of failure (SHR-NF), and from age-matched normotensive Wistar-Kyoto (WKY) rats. Compared to WKY, SHR-NF rats exhibited LV (2.2-fold), and RV (1.5-fold) hypertrophy, while SHR-F rats were characterized by comparable LV hypertrophy (2.1-fold) and augmented RV hypertrophy (2.4-fold; all  $p < 0.01$ ). In SHR-F hearts the level of  $\alpha$ -myosin heavy chain (MHC) mRNA was decreased in both ventricles to 1/3 and 1/5 of the SHR-NF and WKY values, respectively (both  $p < 0.01$ ). Levels of  $\beta$ -MHC, actin, and myosin light chains did not differ among the 3 groups in the LV. Levels of atrial natriuretic factor (ANF) mRNA were elevated 3-fold in the LV of SHR-NF rats ( $p < 0.05$ ), but were not increased in the RV of SHR-NF compared to WKY rats. During the transition to failure, ANF mRNA levels increased an additional 1.6-fold in the LV, and were incremented 4.7-fold in the RV (both  $p < 0.05$ ). The levels of fibronectin (FN), pro- $\alpha 1(I)$  and pro- $\alpha 1(III)$  collagen (CN) mRNAs were not elevated in either ventricle of the SHR-NF group, but were 4-5-fold higher in both ventricles of SHR-F rats (all  $p < 0.05$ ). Transforming growth factor- $\beta_1$  (TGF- $\beta_1$ ) mRNA abundance was not elevated in ventricles of SHR-NF rats but increased 1.3-fold in the LV and 2-fold in the RV during the transition to heart failure (both  $p < 0.05$ ). The decrease in  $\alpha$ -MHC mRNA levels represents a pretranslational basis for the slowed contraction observed in cardiac muscle from failing hearts. The increase in FN and CN mRNA levels suggests that the observed increase in myocardial fibrosis in failing hearts is regulated at the level of gene expression. The increase in abundance of TGF- $\beta_1$  mRNA in conjunction with the upregulation of FN and CN genes suggests that activation of TGF- $\beta_1$  gene expression may be a mechanism initiating interstitial fibrosis during the transition from stable, compensated hypertrophy to overt heart failure.

#### Induction of Immediate-Early Gene by Extracellular ATP in Cardiac Cells

Norepinephrine (NE) activates processes of hypertrophy and proliferation in cardiac myocytes and fibroblasts, respectively, in conjunction with expression of immediate early genes (IEG). Since adenosine 5'-triphosphate (ATP) is co-released with NE from sympathetic nerve endings in the heart, we studied the



effects of extracellular ATP on IEG expression in cardiac cells and investigated the intracellular mechanisms of IEG induction by ATP. In response to micromolar quantities of extracellular ATP, levels of IEG mRNA increased at 15 min, peaked 30 min (5-8 fold), and declined to baseline by 1 hr in both cell types. ATP increased intracellular  $\text{Ca}^{2+}$  concentration ( $\text{Ca}_i$ ) in cardiac myocytes (MYO) and fibroblasts (CAFB) loaded with Indo-1-am, whereas NE did not. The potency order of ATP analogues for increasing  $\text{Ca}_i$  and *c-fos* mRNA levels was:  $\text{ATP} > \text{ADP} > \text{2-met-ATP} > \text{AMP-PNP}$ . Adenosine had no effect on either *c-fos* or  $\text{Ca}_i$  levels. In MYO, the ATP-induced increase in  $\text{Ca}_i$  and *c-fos* were inhibited by pretreatment with intracellular  $\text{Ca}^{2+}$  chelator, BAPTA-AM. However, pretreatment with BAPTA-AM did not inhibit the NE-induced increase in IEG in MYO. NE increased the rate of protein synthesis (incorporation of  $^{14}\text{C}$ -phenylalanine into TCA-precipitable protein) 2-3-fold, whereas ATP did not. In CAFB, overnight pretreatment with TPA or pretreatment with staurosporine for 30 min decreased the ATP-induced level of *c-fos*. Western blot analysis showed that ATP induced tyrosine phosphorylation of a 40-45-kDa protein. These data suggest that the ATP-induced increase in IEG occurs via activation of  $\text{P}_2$ -purinergic receptors in both cell types, and that in CAFB multiple second messenger systems are involved. In MYO, IEG-induction by ATP, but not NE, is calcium dependent, suggesting that ATP and NE activate IEG expression by different intracellular signalling mechanisms. Furthermore, these results suggest that induction of *c-fos* is not sufficient to activate hypertrophy in MYO.

#### Nuclear Transcription and Skeletal and Cardiac Muscle Aging

A significant component of musculoskeletal frailty in aging animals is diminished contractile capacity. The relatively high level of protein accumulation required for the assembly of the contractile apparatus requires specialized systems to selectively stabilize the proteins and mRNA encoding them and a high level of sustained transcriptional activity driven by muscle-specific and ubiquitous nuclear transcription factors. These studies examine three such factors that are present in relatively high levels in both cardiac and skeletal muscle and that are known to drive expression of a number of muscle-specific genes. These are 1) the serum response factor (SRF), 2) a zinc-dependent DNA-binding protein that recognizes G-rich sequences, and 3) the thyroid hormone receptors. The first two factors are involved in the transcription of a number of muscle-specific genes, including sarcomeric actins, the myosin light chains, myoglobin, and the muscle-specific isoforms of creatine kinase. Reagents are being developed to measure their levels of expression in tissues from young and aging animals. The thyroid receptors also act as nuclear factors involved in tissue-specific expression of specific contractile genes. Their importance to aging lies in the fact that, in terms of gene expression, aging resembles a hypothyroid state. Activation of the genes, such as the b-myosin heavy chain, that are associated with hypothyroidism may have important functional consequences. Since numerous have failed to provide a consensus on whether thyroid hormone levels actually change with age, we have focussed on possible changes in the level of expression or the types of receptors expressed during. Our preliminary indication are that there are selective decreases in one thyroid receptor subtype with aging that could account for the changes seen in gene expression. A precise understanding of the molecular mechanisms by which cardiac and skeletal muscle-specific gene expression is initiated and sustained provides the background for understanding age-associated changes in gene transcription that may contribute to musculoskeletal frailty in the aging.



## Involvement of cardiac Opioids in Response of the Heart to Stress

Previous work in the LCS has shown that opioid peptides can directly modulate cardiac myocyte contractile function and inhibit catecholamine induced inotropy by affecting signal transduction through the  $\beta$ -adrenergic pathway. Since both contractile force and  $\beta$ -adrenergic mediated events are involved in the hypertrophic process, we sought to determine whether the cardiac response to hypertrophic stress is associated with changes in opioid peptide levels in rat heart, whether opioid peptide production is regulated at the level of gene expression, and investigated factors that regulate opioid gene expression in the heart. We studied opioid peptide production and/or proenkephalin (PNK) gene expression in 3 well established models of cardiac hypertrophy: aging, aortic constriction and  $\alpha$ -adrenergic stimulation in culture. Cardiac opioid peptide was upregulated 2-3-fold with advancing age, and PNK gene expression was elevated in the heart during aging (7-fold), and during chronic pressure overload hypertrophy leading to heart failure (2-fold). Paradoxically, PNK gene expression was transiently downregulated 3 days after aortic constriction, and this transient decrease was abolished by prior chemical sympathectomy. Norepinephrine (NE) treatment increased the level of PNK mRNA in myocyte cultures (peak 4 hr). Neither  $\alpha$ - nor  $\beta$ -adrenergic receptor stimulation alone was sufficient to produce PNK induction. The induction of PNK by NE in myocytes did not occur unless myocytes were co-cultured with cardiac fibroblasts (in situ hybridization), and could not be reproduced with conditioned media from cardiac fibroblasts. The induction of hypertrophy marker genes (ANF and  $\alpha$ -skeletal actin) by NE in myocyte cultures was not diminished by adding opioid peptides to the media, nor augmented by supplementing the media with opioid receptor antagonists. In summary, opioid peptide production is augmented during chronic cardiac hypertrophy, and is regulated, in part, at the level of gene expression. The negative modulatory influence of opioid peptides on cardiac myocytes may be limited to functional parameters, and apparently does not affect hypertrophic growth of myocytes.

## LABORATORY OF CARDIOVASCULAR SCIENCE VASCULAR INITIATIVE

### Mechanisms for Signal Transduction of Shear Stress Forces in Endothelial Cells

The vascular endothelium, positioned between the flowing blood and the vessel wall, is uniquely exposed to hemodynamic shear stress forces. To study the effect of shear stress forces on vascular endothelial pH and cytosolic  $[Ca^{2+}]_i$ , cells were cultured in 1 mm<sup>2</sup> glass capillary tubes, loaded with the fluorescent indicator carboxy-seminaphtharhodafleur-1 (SNARF-1 for pH) or indo-1 ( $[Ca^{2+}]_i$ ), and studied on the stage of a modified inverted fluorescence microscope. These capillary tubes facilitate pH or  $[Ca^{2+}]_i$  measurements in a closed system which does not allow gas diffusion when using CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>-buffered solutions; small changes in flow rate result in relatively large changes in shear stress forces. We have recently reported that flow-dependent intracellular acidification occurs in endothelial cells during brief exposures to continuous laminar shear stress forces in a physiologic buffer with bicarbonate due to parallel activation of Na<sup>+</sup>-independent Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange and Na<sup>+</sup>/H<sup>+</sup> exchange (Science 258: 656-659, 1992). This change in intracellular pH (pH<sub>i</sub>) is sustained during a 30 minute exposure to shear stress forces of 13.4 dyne cm<sup>-2</sup>, although partial recovery from the acidification occurs during a 30 minute exposure to shear stress forces of 2.7 dyne cm<sup>-2</sup> or less. To determine the mechanism of the partial recovery of pH<sub>i</sub> during a 30 minute exposure to shear stress forces of 2.7 dyne cm<sup>-2</sup> or less, cells



were exposed to ethylisopropylamiloride (EIPA), a  $\text{Na}^+/\text{H}^+$  exchange inhibitor or to  $\text{Na}^+$ -free buffer to inhibit  $\text{Na}^+$ -dependent exchange mechanisms. While EIPA had no effect, removal of buffer  $\text{Na}^+$  significantly inhibited the  $\text{pH}_i$  recovery. These results suggest that while  $\text{Na}^+$ -dependent  $\text{Cl}^-/\text{HCO}_3^-$  exchange is also activated by hemodynamic shear stress exposure. Studies were also performed to characterize the  $\text{pH}_i$  response following a 30 minute exposure to shear stress forces. After return to control conditions, a slowly-developing increase in endothelial  $\text{pH}_i$  of approximately 0.20 pH units has been noted on return to control conditions. Following this alkalization,  $\text{pH}_i$  recovers to control values over 15-20 minutes. Thus,  $\text{pH}_i$  appears to play a significant role in the response of the vascular endothelium to shear stress forces.

#### Effect of Free Radicals on Endothelial Cell $\text{Ca}^{2+}$ Homeostasis

Free radicals are major determinants of oxidant injury during inflammation and post-ischemic reperfusion. Vascular endothelium is both a source and a target of reactive oxygen species. One such reactive oxygen species, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) may cause endothelial cell damage by affecting intracellular signal transduction mechanisms and disrupting  $\text{Ca}^{2+}$  homeostasis. The aim of the project is: 1) to characterize the effects of exposure of human aortic endothelial cells (HAEC) and human umbilical vein endothelial cells (HUVEC) to  $\text{H}_2\text{O}_2$  in the range 0.1mM-1mM on endothelial cell  $\text{Ca}^{2+}$  homeostasis; 2) to define the role of cytosolic  $\text{Ca}^{2+}$  concentration,  $[\text{Ca}^{2+}]_i$ , in the pathophysiology of oxidative injury. In order to detect the effect of hydrogen peroxide on HAEC and HUVEC  $[\text{Ca}^{2+}]_i$ , indo-1 AM loaded endothelial cells grown on glass coverslips were bathed in a Hepes buffer ( $\text{CaCl}_2$ , 1.5mM, pH 7.4) containing  $\text{H}_2\text{O}_2$  in the range 0.1mM-1mM.  $\text{H}_2\text{O}_2$  slowly increased  $\text{Ca}_i$  and this effect was not reversible with wash-out. The increase in  $[\text{Ca}^{2+}]_i$  persisted in a  $\text{Ca}^{2+}$ -free solution with 1mM EGTA suggesting that extracellular  $\text{Ca}^{2+}$  does not contribute to the increase in  $[\text{Ca}^{2+}]_i$ . In other experiments the effect of  $\text{H}_2\text{O}_2$  on endothelial cell  $[\text{Ca}^{2+}]_i$  was examined following exposure to 1 $\mu\text{M}$  bradykinin, an agonist known to release  $\text{Ca}^{2+}$  from intracellular stores. Under these conditions,  $\text{H}_2\text{O}_2$  still produced an increase in  $[\text{Ca}^{2+}]_i$ . Pre-exposure of HAEC to thapsigargin, an inhibitor of microsomal or endoplasmic reticulum (ER)  $\text{Ca}^{2+}$ -ATPase which releases  $[\text{Ca}^{2+}]$  from the endoplasmic reticulum, abolished the response to bradykinin (1 $\mu\text{M}$ ) but did not suppress the subsequent response to  $\text{H}_2\text{O}_2$ . These results suggest that  $\text{H}_2\text{O}_2$  releases  $[\text{Ca}^{2+}]$  from a thapsigargin-insensitive intracellular store and increases  $[\text{Ca}^{2+}]_i$  via this mechanism.

#### A novel Cardiac Myofilament Desensitizing Substance Released by Endothelial Cells

Vascular endothelium regulates smooth muscle tone through the coordinated release of agents such as prostacycline, endothelium-derived relaxing factor (EDRF) or nitric oxide, and endothelin. Recent studies, both in isolated cardiac multicellular preparations and in intact hearts in vivo, indicated that endocardial endothelium may similarly influence myocardial contraction predominantly by modulating the onset of relaxation. Bioassay studies using cultured endocardial endothelial cells and isolated cardiac papillary muscle preparations suggest that diffusible agents are involved, one of which is probably nitric oxide. However, the relative contribution of endocardial endothelial and coronary vascular endothelial cells to these effects, and the myocardial mechanism of action of substances released by these cell types is unclear. We studied the effects of effluent of superfused endocardial and vascular endothelial cultured cells on contraction and intracellular calcium





transients of isolated adult rat cardiac myocytes. Both endocardial and vascular endothelial cells tonically released a novel substance which rapidly and reversibly decreased the amplitude of myocytes twitch contraction by inducing earlier relaxation, and also increased diastolic cell length. These effects were not associated with any change in the intracellular calcium transient, indicating cardiac myofilament "desensitization". The activity of endothelial cell effluent remained stable at 37°C for several hours or at 4°C for at least 48 hours. The action of this substance did not involve nitric oxide, cyclic GMP or prostanoids, nor changes in intracellular pH. These properties suggest that endothelial cells may rapidly modulate cardiac contraction-relaxation coupling and diastolic tonus by altering myofilament properties, as well as exert distant effects because of the unusual stability of this substance.

#### Angiogenesis Endothelial Cell Function and Aging

Angiogenesis (the formation of new blood vessels) is an important process in tumor growth, wound healing, retinopathies, and rheumatoid arthritis. We are investigating aspects of angiogenesis in an in vitro model utilizing isolated human and bovine endothelial cells and a mixture of basement membrane matrix proteins (Matrigel) or collagen gel.

Normally endothelial cells are quiescent in vivo; but in response to an angiogenic factor, activated endothelial cells will breakdown their underlying basement membrane, migrate into the interstitium, proliferate, and finally differentiate into a new blood vessel. We found that quiescent endothelial cells (contact inhibition or serum starvation) were not able to organize into a capillary-like network in vitro, while cells released from contact inhibition or feed serum-containing media regained the ability to organize. There was a correlation between the ability to organize on Matrigel and the expression of cell surface receptors for the basement membrane proteins, laminin and collagen IV. These studies should prove useful in studying some early events involved in angiogenesis.

Angiogenic factors attract leukocytes and fibroblast in an area, while stimulating the formation of new blood vessels. We tested whether fibroblast produced some factor that altered the ability of endothelial cells to organize into new vessels. We found that fibroblast-conditioned media contained a cellular attractant and enhanced the extent of capillary-like formations on Matrigel or within a collagen gel. The ability of fibroblast to influence angiogenesis may be an important component of wound-healing.

We found that TGF- $\beta$  caused differentiated endothelial cells on Matrigel or on collagen 1 gels to undergo apoptosis. TGF- $\beta$  may be important for the final stages of wound healing when the area forms scar tissue and becomes avascular.

#### Role of Vascular Smooth Muscle Cells in Vascular Disease

The migration, proliferation, and neointimal accumulation of vascular smooth muscle cells (VSMCs) are key events in the development and progression of many vascular diseases and a predictable consequence of mechanical injury to the blood vessel. VSMCs in vivo are surrounded by and embedded in extracellular matrices (ECMs) that must be traversed during migration. In many other cell types, migration across ECM barriers involves the local destruction or degradation of these barriers by extracellular proteases. Principle among such proteases are



those belonging to the matrix metalloproteinase (MMP) family. Using an *in vitro* assay to monitor and manipulate the ability of VSMCs to degrade a defined ECM barrier as they migrate toward a chemoattractant, we demonstrate the VSMCs isolated from the rat thoracic aorta and maintained in a proliferating or "synthetic" state readily migrate through an ECM barrier of reconstituted basement membrane. The migration of serum-starved/differentiated VSMCs toward the chemoattractant both in the presence and in the absence of the barrier is less than 20% ( $p < 0.001$ ) that of proliferating cells. The importance of MMP expression during the migration of "synthetic" VSMCs through the reconstituted BM was demonstrated using a peptide that mimics the inhibitory propeptide region of all MMPs. This peptide blocked migration of proliferating cells through the barrier by more than 80% ( $p < 0.005$ ), but did not significantly affect migration that occurred in the absence of the barrier. Likewise, antisera capable of neutralizing the activity of the 72 kD type IV collagenase (MMP-2) also inhibited migration through the barrier, without significantly affecting the migration of cells in the absence of the barrier. Northern blotting and zymographic analyses indicate that MMP2 is the principal MMP expressed and secreted by these cells. MMP2 activity expressed by serum starved/differentiated VSMCs as measured by a fluorescent peptide cleavage assay was less than 5% of that measured in proliferating VSMCs. These results demonstrate that VSMCs migrate through an ECM barrier similar in composition to one that normally surrounds them and that this ability is regulated by the phenotypic state of the cell.

#### Role of Age in Vascular Smooth Muscle Cell Migration and Invasion

The development and progression of many vascular diseases depend on the migration and proliferation of vascular smooth muscle cells (VSMC) and their interaction with extracellular matrix (ECM). The incidence and prevalence of vascular disease increase with age, affecting approximately 50% of men (by age 65) and women (by age 75). We have found previously that proliferating VSMC aggressively degrade and invade a reconstituted basement membrane barrier (modeled to mimic the basement membrane which surrounds individual VSMC and separates them from endothelial cells in the form of the internal elastic lamina *in vivo*) in response to PDGF, while the migration and invasion of serum-starved/differentiated VSMC was less than 20% ( $p < 0.001$ ) that of proliferating cells. We demonstrated that VSMC migration through this ECM barrier requires 72 kD Type IV gelatinase. In this project we investigated the migratory/invasive, proliferative, and differentiative behavior of VSMC derived from young (age 3-6 mo) and old (age 24 mo) rats. Two populations of VSMC (neointimal and medial) were obtained following balloon catheter injury for each age group. Early passage ( $P_2$ - $P_3$ ) young neointimal VSMC exhibit 75% more migratory and invasive behavior as compared with ( $P_2$ - $P_3$ ) young medial VSMC. At later passages ( $P_6$ - $P_{16}$ ) young medial and young neointimal VSMC exhibit similar migratory and invasive characteristics. In contrast, old medial ( $P_2$ - $P_3$ ) show as aggressive migratory and invasive behavior as old neointimal ( $P_2$ - $P_3$ ). When VSMC from all four groups were growth arrested, their migratory and invasive behavior was less than 20% that of age/phenotype matched proliferating cells. We have observed differences in active 72 kD Type IV gelatinase and in receptor tyrosine kinase (RTK) activation in response to PDGF between proliferating and differentiated VSMC. Future studies employing gene markers of differentiated and proliferating VSMC such as calponin, CHIP 28 and Osteopontin as well as 72 kD Type IV gelatinase expression and activation should provide important information in understanding these age-associated behavioral differences in migratory/invasive behavior of VSMC.



### Signal Transduction Pathways Involved in Vascular Smooth Muscle Cell Migration

The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular diseases. We have previously shown that in vitro VSMC migration in response to PDGF is suppressed in differentiated VSMCs and seek to identify differences in intracellular signalling between differentiated and proliferating VSMCs that may account for this suppression. Differentiated VSMCs retain their ability to respond to PDGF and upregulate expression of the immediate early response genes, *c-fos* and *MCP-1* (JE) when stimulated by PDGF. Unlike proliferating cells, however, PDGF-stimulated differentiated VSMCs fail to activate calcium/calmodulin-dependent protein kinase (CamKinase) II activity. Blocking CamKinase II activation blocked the migration of proliferating VSMCs by more than 90%. In contrast, inhibitors of protein kinase C have no significant effect on migration. Pretreatment of differentiated cells with ionomycin (1  $\mu$ M) or endothelin (10-100 nM) (both of which are expected to increase intracellular calcium) resulted in an 84 + 6% return to the migration rate of proliferating VSMCs. This return was also blocked by CamKinase inhibitors and was unaffected by inhibitors of PKC. These results suggest that activation of CamKinase plays an important role in VSMC migration and the failure to activate it in differentiated VSMCs may be responsible for the suppression of migration. The most direct intracellular pathway by which PDGF could activate CamKinase II is through the activation of phospholipase C $\gamma$  (via tyrosine phosphorylation) following its association with the activated PDGF receptor. Immunoprecipitation of PLC $\gamma$  followed by blotting of the protein with an antibody to phosphotyrosine residues indicates that, in contrast to proliferating VSMCs, differentiated VSMCs do, in fact, fail to activate PLC $\gamma$ . Recent experiments have identified a role for basic FGF in the migration of VSMCs and suggest that PLC $\gamma$  activated by PDGF may be indirect and mediated through the FGF receptor. Our results focussing on PDGF intracellular signalling have identified at least one critical difference in the way in which proliferating and differentiated VSMCs respond to PDGF and have demonstrated that to activate differentiated VSMCs requires concurrent action by at least two growth factors/cytokines. This requirement may limit the response of VSMCs to injury to selected group capable of responding to agents.

### Vascular Smooth Muscle Gene Expression and Cellular Differentiation

The phenotypic modulation of vascular smooth muscle cells (VSMCs) from a quiescent, differentiated state to that of proliferating, "undifferentiated" cells is a common pathogenic feature of vascular disease. The molecular mechanisms by which this phenotypic "switch" is achieved are unknown. We have observed that proliferating vascular smooth muscle cells express mRNAs that are homologous to a gene expressed in other tissues that acts as a transdominant suppressor of differentiation. This gene is referred to as ID (inhibitor of differentiation). It belongs to the family of helix-loop-helix (HLH) family of proteins, many of which are involved in cell determination and differentiation. Using a variety of molecular cloning techniques, we have further identified and cloned two ID-like cDNAs from proliferating VSMCs. One of these is the rat homologue of mouse ID1 (i.e. rat ID1) and the other is apparently a unique member of the ID family. We have further demonstrated that rat ID1 exists as two protein isoforms which are the products of alternative RNA splicing of the rat ID1 gene. Expression of rat ID1 is upregulated by a number of growth factors that are important in initiating and sustaining VSMC proliferation, such as PDGF, bFGF, and IGF-1. Expression of rat ID1 is often (but not always) downregulated when VSMCs are made quiescent. However, when cultured VSMCs achieve a level of



differentiation comparable to that in the intact vessel (for example, when they are cultured on reconstituted basement membrane), ID expression decreases 10-fold to a level similar to that seen in the intact, uninjured vessel. Our group is currently initiating studies to identify the function of ID in VSMC migration, proliferation, and differentiation by overexpressing rat ID1 from constitutively active and inducible promoters, by antisense inhibition of rat ID1, and by genetic screening of cDNA libraries using the two hybrid yeast system to identify potential protein partners for rat ID1.

#### Molecular Characterization and Therapeutic Interventions for Restenosis

Percutaneous Transluminal Coronary Angioplasty (PTCA) has become a widely used procedure for the treatment of coronary artery disease (CAD). However, restenosis at the site of PTCA remains a persistent problem. Restenosis following PTCA involves a fibroproliferative response to vascular injury and numerous attempts to modify this response, either through pharmacological interventions or mechanical devices, have met with very limited success. It is felt that by understanding the cellular and molecular mechanisms that underlie this fibroproliferative response to injury, more effective strategies to prevent restenosis can be explored. One mechanism that has been shown to be important in restenosis in the rat is the migration of vascular smooth muscle cells (VSMCs) from the media of the vascular wall to the intima. Recent studies by LCS scientists in cultured VSMCs suggest that the MMP-2 Type IV metalloproteinase (MMP-2) may be important in this process. Using gelatin zymography on extracts from carotid artery tissue isolated at varying time points following injury, we previously demonstrated the presence of the MMP-2 protein. To further clarify the in vivo relevance of this enzyme to the arterial response to injury, experiments involving the rat carotid injury model were performed. Deendothelialization was performed with a embolectomy catheter and vessels were subsequently harvested at the appropriate time points. Preliminary findings with immunocytochemistry, in situ hybridization studies, and the ribonuclease protection assay reveal parallel results. The mRNA and protein for the MMP-2 gene was expressed at moderate levels in uninjured vessels and decreased in expression at early time points. Within 5-7 days following injury expression increased and peaked at 14-21 days. The functional significance of these changes in vivo is presently under investigation. Treatment for restenosis is dependent on the development of an effective means of intravascular site-specific delivery. We recently began studies using a unique sustained-release biodegradable microcapsule. Initial experiments using Texas-red labeled albumin (TRA) incorporated into the microcapsules showed penetration of the TRA into the media in vessels from which the adventitia had been removed. Based upon preliminary results obtained about the potential role of MMP-2 in VSMC migration, we initiated in vivo studies using a peptide inhibitor of metalloproteinase activity. The peptide inhibitor was incorporated into the microcapsule and applied to the outside of the rat carotid artery that had undergone vascular injury. The results of these experiments are pending.

#### Treatment of Restenosis After Angioplasty with Microtubule Stabilizing Agents

Significant improvements in the primary success rate of various medical and surgical treatments of atherosclerotic disease have been made in the last few years. Yet recurring failures continue in 30 to 50% of the patients after balloon angioplasty, bypass surgery, and enterectomy because of late restenosis of the treated vessel. The restenosis is a result of a complex series of





fibroproliferative responses to the vascular injury that results in vascular smooth muscle cell (VSMC) proliferation, migration, neointimal accumulation, and secretion of extracellular proteins. Microtubules are likely involved in controlling or moderating critical intracellular mechanisms necessary for the VSMC fibroproliferative response. We found that taxol, an anti-tumor drug which stabilizes microtubules, inhibited VSMC proliferation, migration, and invasion *in vitro*. *In vivo*, taxol prevented neointimal VSMC accumulation in the rat carotid artery after balloon dilation and endothelial denudation injury. These experiments suggest that taxol or other pharmacologic agents that stabilize microtubules may have therapeutic value in preventing human restenosis after balloon angioplasty, bypass surgery, and enterectomy.

#### Gene Therapy of Coronary Artery Disease

Gene therapy may represent a novel approach for the treatment of myocardial ischemia. This research project aims at developing adenoviral vectors to transfer the cDNA for endothelial cell growth factors into cardiac cells. The same adenoviral vectors will be used for two different studies: (1) Angiogenesis and improvement of coronary collateral circulation: Neovascularization is expected to improve blood flow to ischemic areas of the myocardium. For this study the adenoviral vectors will be injected into the coronary circulation or directly into the myocardium. (2) Restenosis after angioplasty: Rapid reendothelialization of a segment of coronary artery which has undergone endothelial denudation during angioplasty may be expected to decrease the severity of restenosis and intimal hyperplasia. For this study the adenoviral vectors will be delivered to the localized area of the coronary artery which has undergone balloon dilatation. We have constructed adenoviral vectors which carry the cDNA for the following angiogenic growth factors. (1) Vascular endothelial growth factor (VEGF). (2) Acidic fibroblast growth factor (aFGF). (3) A recombinant form of aFGF which has been modified with the addition of the secretory signal sequence from FGF-4 (sp-aFGF). Unlike the natural form of aFGF this recombinant form of aFGF is secreted into the extracellular space.

Our initial studies show all three adenoviral vectors produce a functional protein capable of inducing endothelial cell growth and differentiation *in vitro*. In additional studies with an adenoviral vector which carries the cDNA for the reporter gene lacZ (AdRSV.lacZ) we have examined whether adenoviral vectors can transduce cardiac cells in the minipigs. We have found that intracoronary injection of Ad RSV.lacZ transduces endothelial cells, vascular smooth muscle cells and myocardial cells. In contrast, intramyocardial injection of AdRSV.lacZ transduces mostly myocardial cells. Studies are now in progress to further characterize the properties of the vectors which carry the cDNA for the angiogenic factors prior to their use in *in vivo* models of myocardial ischemia and restenosis after angioplasty.

#### Hormonal Requirements for Intimal Thickening Following Vascular Injury in Rats

The fibroproliferative response of vascular smooth muscle cells (VSMCs) to injury is regulated by the combined action of both autocrine and paracrine growth factors. In addition, hormonal factors circulating in the blood plasma are important for cellular growth of VSMCs in tissue culture. *In vivo*, a similar paradigm seems to exist after vascular injury where growth factors that are locally produced act in conjunction with plasma factors to induce proliferation. It has been shown that hormonal factors dependent on the pituitary gland are



involved in VSMC proliferation and migration after arterial injury. Injury-induced neointimal formation is inhibited in hypophysectomized rats. Indeed, atherosclerosis or restenosis may in part be mediated by a complex endocrine modulation.

Hypophysectomy is a severe intervention and causes alterations in a number of hormonal factors; therefore, the exact mechanism(s) or factor(s) dependent on the pituitary gland is not known. In an attempt to further clarify this issue and pinpoint specific hormonal factors necessary for intimal thickening, we made rats specifically hypothyroid by dietary manipulations. Preliminary experiments show that rats fed thyroid suppressive diets (thiouracil-containing) exhibit a markedly diminished response to neointimal formation following balloon angioplasty. This blunted response was observed at 8, 14 and 21 days following vascular injury with the most significant inhibition occurring at 21 days. This also occurred in animals which, in addition to being rendered hypothyroid were made hyper-cholesterolemic. These observations suggest that the anti-proliferative effect of hypophysectomy on neointima formation is in part specifically mediated by a deficiency in thyroid hormone.

#### Age-Associated Changes in Vascular Stiffness Properties

In industrialized societies increases in arterial stiffness and left ventricular (LV) mass are considered part of normative aging. However, considerable heterogeneity exists in the age-associated changes in these variables both across cultures as well as within a given population. The ultimate goals of this project are to address the issue of how alterations in arterial stiffness affect the myocardium and whether in outcome studies they relate to vascular insufficiency syndromes, e.g., stroke. We have initiated a pilot study in which measurements of cardiac mass (via NMR), filling properties and isovolumic relaxation time (via Doppler echocardiography), carotid pressure pulse (via applanation tomography) and arterial pulse wave velocity (Doppler sonography) are made in men and women who differ with respect to age, race, arterial pressure, body composition, and physical conditioning status. As expected, results indicate that with increasing age arterial stiffening leads to an increase in pulse wave velocity. This is associated with an early return of reflected pulse waves from peripheral sites which produce an augmented and late occurring peak of carotid pressure pulse. It is important to note that these arterial changes are, by and large, not detected by routine clinical measures of brachial arterial pressure and are independent of gender, but vary inversely with exercise capacity. Of note, also is that in highly physically conditioned older individuals (>60 yrs of age) the arterial stiffness and reflected wave indices are markedly reduced relative to their sedentary age peers and do not differ from those of younger individuals, although the age-associated increase in systolic pressure persists. We have undertaken an initiative to extend many of these studies to Black Americans and to other non-Western populations in which different patterns of arterial pressure change occurs with aging (in China via a research contract (cf. #N01-AG-02-2118)). Long term goals include determination of whether "normative" age-associated changes in cardiac structure (i.e. increasing left ventricular mass) and function (delayed left ventricular relaxation and reduced early diastolic filling rates) are directly related to changes in arterial stiffness. If such is the case, a long-term intervention trial might be justified to see whether amelioration of arterial stiffness by medication or lifestyle intervention can attenuate these age-associated changes in cardiac structure and function and reduce future cardiovascular morbidity



### Vascular Smooth Muscle Cell Function and Age-Related Hypertension

Peripheral vascular resistance becomes elevated with age increasing the risk of cardiovascular disease. The physiologic changes in the vasculature that accompany aging closely resemble those seen in younger individuals with essential hypertension. The etiology of this condition may involve a defect in intracellular  $\text{Ca}^{2+}$  metabolism. We investigated this hypothesis in a rat model for hypertension using freshly-isolated arterial smooth muscle cells (SMC) which retain their *in vivo* phenotype. Mean systolic blood pressure showed a significant elevation between 6 and 30 months of age (131 mm vs 155 mm;  $p < .001$ ). Old SMC showed transiently higher resting intracellular  $\text{Ca}^{2+}$  levels compared to young cells following exposure to different extracellular  $\text{Ca}^{2+}$  loads. Blockade of T-type  $\text{Ca}^{2+}$  channels by  $\text{Ni}^{2+}$  was more effective in lowering resting  $\text{Ca}^{2+}$  in young than in old SMC, whereas no age difference was found with respect to  $\text{Ca}^{2+}$  sequestration by sarcoplasmic reticulum (SR). Stimulation of SMC with the  $\beta$ -agonist isoproterenol caused the redistribution of  $\text{Ca}^{2+}$  from the SR to the extracellular compartment. Measurements of the rate of isoproterenol-dependent  $\text{Ca}^{2+}$  removal from the SR showed that SMC from old rats retain significant levels of  $\text{Ca}^{2+}$  over longer periods of time than young SMC. Increased SR  $\text{Ca}^{2+}$  stores following  $\beta$ -agonist stimulation could result in diminished smooth muscle relaxation and increased vascular tonus contributing to the development of hypertension in older animals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00226-11 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Excitation-Contraction Mechanisms in Isolated Cardiac Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. M. Janczewski	Visiting Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	H. A. Spurgeon	Research Physiologist	LCS, NIA
	K. Bogdanov	Guest Researcher	LCS, NIA

COOPERATING UNITS (if any)

Johns Hopkins University, Division of Cardiology (M. Stern)  
University of Turku, Department of Biology (A. Talo)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The coupling of surface membrane depolarization of heart cells to contraction is mediated by a release of  $Ca^{2+}$  from the sarcoplasmic reticulum (SR). The precise mechanism of coupling of the depolarization to SR  $Ca^{2+}$  release is presently the preeminent issue in cardiac biophysics. Morphological studies of cardiac cells have shown that the junctional SR, from which  $Ca^{2+}$  is released upon stimulation, forms structural "couplings" with the sarcolemma (SL), primarily (by 80%) with the T tubules, i.e. invaginations of the SL into the cell interior. At the "couplings", the SL  $Ca^{2+}$  channels and the SR  $Ca^{2+}$  release channels span the gap between closely apposed but separate SL and SR membrane systems. It has been generally postulated, therefore, that a direct regulatory effect of the L-type  $Ca^{2+}$  current ( $I_{Ca}$ ) on the SR  $Ca^{2+}$  release, typical for mammalian ventricular myocytes, results from a preferential access of the  $Ca^{2+}$  current to the SR  $Ca^{2+}$  release sites via the "couplings". The present project is aimed on testing this hypothesis by comparing the dependence of SR  $Ca^{2+}$  release on the L-type  $Ca^{2+}$  current in mammalian (rat) ventricular myocytes, which have an extensive T tubular system and extensive "couplings", and avian (Finch) ventricular myocytes, which lack the T tubules and, therefore, over 80% of its junctional SR lacks contact with the SL. Rat and Finch ventricular cells are isolated from the heart (collagenase digestion), loaded with a  $Ca^{2+}$  indicator (indo-1/FA) via a patch pipette, and voltage clamped (whole cell patch method). Test depolarizations lasting 25 to 250 msec to potentials that span the range of the L-type  $Ca^{2+}$  channel activation are then made and  $I_{Ca}$  and  $Ca^{2+}$  transient amplitude are measured. The results obtained so far show that in bird ventricular myocytes the SR  $Ca^{2+}$  release is well correlated to  $Ca^{2+}$  influx via the L-type  $Ca^{2+}$  channels and is qualitatively similar to the relationship between  $I_{Ca}$  and the SR  $Ca^{2+}$  release observed in rat ventricular myocytes. Thus, the results suggest that the direct control of SR  $Ca^{2+}$  release by  $I_{Ca}$  is not attributable to a preferential access of  $Ca^{2+}$  influx to SR  $Ca^{2+}$  release sites via the SL-SR "couplings". This apparently necessitates a change of the current dogma regarding the functional significance of these "couplings". Specifically, models of excitation-contraction coupling in which  $Ca^{2+}$  diffusion is more extensive perhaps in series with local control models (20% of coupling in Finch) will need to be explored.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00228-10 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age-Associated Changes in Cardiac Rhythm and Conduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Fleg Unit Chief LCS, NIA  
Others: R. Mayuga Guest Researcher LCS, NIA  
F. O'Connor Statistician (Health) LCS, NIA  
C.T. Arrington Mathematical Statistician (DOD 3/17/93) LCS, NIA

COOPERATING UNITS (if any)

University of Maryland, College Park (E. Byrne, S. Porges); Rush Medical College, Chicago (Harold Kennedy).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

**A.** In subjects without known organic heart disease, ventricular arrhythmias precipitated by exercise or occurring during routine activity increase in frequency and complexity with age and cause substantial cardiac morbidity. Although increased echocardiographic left ventricular (LV) mass predicts a higher prevalence and complexity of ventricular arrhythmias (VA) on ambulatory ECG, whether such a relationship between LV anatomy and exercise-induced VA (EIVA) is unknown. We therefore examined this question in 288 healthy BLSA volunteers 20-90 years old who underwent both M-mode echocardiography and maximal treadmill exercise testing. Simple, i.e. isolated, EIVA occurred in 53 subjects (18%) and complex EIVA (comparing  $\geq 10\%$  of beats in any minute or occurring in runs) in 15 subjects (5%). Although univariate predictors of any EIVA were greater LV mass ( $p=.0006$ ), older age ( $=.0009$ ), larger body surface area ( $p=.003$ ), higher peak systolic blood pressure ( $p=.01$ ) and male sex ( $p=.01$ ), only age ( $p=.002$ ) independently predicted EIVA by multiple logistic regression analysis. **B.** Although respiratory sinus arrhythmias (RSA) is known to decrease with advancing age, the independent effects of age, gender, conditioning status and body composition are unknown. The impact of age, fitness, gender and relative weight on resting heart rate variability was examined in 117 healthy normotensive adults ages 19-82 from the BLSA; heart rate variability was indexed by RSA, extracted from a 3-minute seated ECG using time domain digital filtering. By linear regression analysis, RSA varied inversely with age ( $r = -0.61$ ,  $p < .001$ ) and body mass index ( $r = -0.31$ ,  $p < .01$ ), directly with  $VO_{2max}$  ( $r = 0.40$ ,  $p < .001$ ) and was unrelated to gender. Multiple regression analysis demonstrated that age and body mass but not  $VO_{2max}$  were independent predictors of RSA. **C.** To examine the hypothesis that heart rate variability (HRV) is reduced in apparently healthy subjects with latent coronary artery disease (CAD), we measured resting ECG R-R interval variations in 29 asymptomatic male volunteers aged  $61.8 \pm 11.2$  years, free of clinical heart disease, who developed a coronary event (CE), within the next 2 years. Compared to 58 age-matched controls (C) who remained event free for  $12.7 \pm 6.5$  years after the index ECG, men destined for a CE showed smaller standard deviation of R-R ( $26.1 \pm 10.8$  vs  $37.9 \pm 24.0$  msec,  $p = .002$ ) and smaller absolute difference between minimum and maximum R-R ( $98.6 \pm 40.3$  vs  $136.7 \pm 81.0$  msec,  $p = .004$ ) indicating reduced HRV in men with latent CAD.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00231-09 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Energy Metabolism in Aging and Disease: Cardiovascular System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Hansford	Chief, EMBS	LCS, NIA
	F. Di Lisa	Visiting Associate (DOD 11/92)	LCS, NIA
Others:	B. Hogue	Chemist	LCS, NIA

COOPERATING UNITS (if any)

Cardiac Function Section, LCS; Division of Cardiology, Johns Hopkins University (H. Silverman, M. D. Stern)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Energy Metabolism and Bioenergetics Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.7

OTHER:

0.2

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise tissue survival. (1) We have focused this year on the relation between altered mitochondrial  $\text{Ca}^{2+}$  ion homeostasis and the potential for oxidative phosphorylation. We have extended our previous work on cardiac myocytes from the cardiomyopathic Syrian hamster. Studied at the point of failure, these hearts perform less work and also fail to activate pyruvate dehydrogenase as completely as in healthy hearts. In the previous year we tied this to a failure to elevate intramitochondrial free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_m$ ) in cells from myopathic animals, when  $[\text{Ca}^{2+}]_m$  was studied in individual cardiac myocytes subjected to an electrical pacing regimen. This year we showed that the likely cause of the diminished response of  $[\text{Ca}^{2+}]_m$  to increased frequency of electrical stimulation in the cardiomyopathic is the generation of smaller systolic transients in cytosolic free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) in myocytes from the failing hearts. (2) We have extended the previous year's studies on the dependence of  $[\text{Ca}^{2+}]_m$  upon frequency of electrical stimulation, to separate an effect due to  $\beta$ -adrenergic stimulation from that of frequency. Further, we have used the effect of mitochondrial uncoupling agents to provide an independent verification of our finding that the mitochondrial  $\text{Ca}^{2+}$  gradient may be positive or negative, depending upon the degree of cell stimulation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00249-07 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Subcellular Calcium Ion Homeostasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Hansford	Chief, EMBS	LCS, NIA
Others:	I. Kudryashova	Visiting Fellow	LCS, NIA
	B. Hogue	Chemist	LCS, NIA

COOPERATING UNITS (if any)

Cardiac Function Section, LCS; Department of Oncology, Johns Hopkins University (Dr. R. Tucker), Department of Biochemistry, University of Campinas, Brazil (Dr. L. Pereira da Silva)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Energy Metabolism and Bioenergetics Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

0.9

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

This project focuses on the mechanism whereby cells achieve homeostasis of  $Ca^{2+}$  ion concentration, both within the cytosol and other cellular compartments, and allow changes in  $Ca^{2+}$  in response of hormones and neurotransmitters. We are currently focussing on the role of deranged  $Ca^{2+}$  homeostasis in the process of cell death. This year we have investigated the effect of the overexpression of the bcl2 oncogene in the Jurkat T cell line upon regulation of cytosol  $[Ca^{2+}]$  and the thapsigargin-sensitive pool of endoplasmic reticulum  $Ca^{2+}$ . In response to serum-starvation, the bcl2-transfected cells survive longer and maintain a larger thapsigargin-releasable pool of  $Ca^{2+}$  than do the control cells. There may be a cause-and-effect relationship between these two parameters.

We have also asked the question of whether killing of cells by exposure to visible light in the presence of porphyrins involves the peripheral benzodiazepine receptor in the mitochondrial membrane, the opening of the mitochondrial "megachannel" and the consequent release of a pulse of  $Ca^{2+}$  into the cytosol. In studies with mitochondria isolated from heart and liver, exposure to porphyrins and light was found to be extremely effective in inhibiting mitochondrial  $Ca^{2+}$  uptake and in causing release of accumulated  $Ca^{2+}$ . However, the effect was not blocked by cyclosporin, which inhibits the opening of the megachannel, and did involve uncoupling, as shown by fluorescence of the membrane potential-sensitive dye JC-1. Thus, the induction of damage by photodynamic therapy is broader in mechanism than the opening of this channel alone. These studies on  $Ca^{2+}$  in cell death are geared towards an understanding of the basic biology of this process, with an eventual goal of designing interventions to prevent unwanted cell death in fixed, post-mitotic tissues during aging.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00259-05 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Signal Transduction of Opioid Receptor Stimulation of Myocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. Lakatta Chief LCS, NIA

Others: R-P. Xiao Visiting Associate LCS, NIA  
H. Spurgeon Research Physiologist LCS, NIA  
M. Capogrossi Medical Officer LCS, NIA

COOPERATING UNITS (if any)

Department of Biochemistry, University of Bologna Medical School, Bologna, Italy.

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)

The recent discovery of the presence of opioid peptide receptors on cardiac ventricular cells has prompted investigation of functional effects that result from stimulation of these receptors. In this regard, a naturally occurring opioid peptide, Leucine enkephalin (Leuenk), a  $\delta$  receptor agonist, leads to a marked reduction in the twitch amplitude of single adult rat ventricular myocytes. This effect is due, in large part, to a reduction in the amplitude of the cytosolic  $Ca^{2+}$  transient ( $Ca_i$ ). The  $Ca_i$  transient following the excitation of heart cells is due to activation of L-type sarcolemmal  $Ca^{2+}$  channels leading to  $Ca^{2+}$  influx via these channels ( $I_{Ca}$ ). This  $Ca^{2+}$  influx triggers  $Ca^{2+}$  release from the sarcoplasmic reticulum (SR) and also loads the SR with  $Ca^{2+}$  for subsequent releases. The specific mechanism by which  $Ca_i$  is reduced by Leuenk have been partly elucidated. Leuenk causes a release of  $Ca^{2+}$  from the SR and leads to a reduction in the amount of  $Ca^{2+}$  in the SR stores. These effects may be attributable to an increase in  $IP_3$  and  $IP_2$  produced by Leuenk. However, it is unknown whether Leuenk also decreases  $I_{Ca}$ . In this regard, the effect of opioid peptides to block neurotransmitters release from neurons has been attributed to a reduction in  $Ca^{2+}$  channel current in these cells. In the present study we determined the effect of Leuenk on  $I_{Ca}$  of individual cardiac ventricular cells freshly isolated from adult rats.  $I_{Ca}$  was measured via patch pipette in the whole cell voltage clamp mode. We observed that Leuenk ( $10^{-6}M$ ) decreases the amplitude of  $I_{Ca}$  by 40% during regular stimulation at 0.2 Hz at 23°C. Thus the  $I_{Ca}$  IV relation was not altered by Leuenk. The  $I_{Ca}$  depression by Leuenk was abolished by Naloxone, a specific  $\delta$  receptor antagonist. The  $I_{Ca}$  inactivation kinetics were unaffected by Leuenk. That Leuenk decreases the magnitude of  $I_{Ca}$  indicates that stimulation of  $\delta$  opioid receptors leads to both a reduction in the magnitude of the trigger for  $Ca^{2+}$  release and to a reduction in  $Ca^{2+}$  loading of the SR. Thus, the opioid peptide effects to decrease the  $Ca_i$  transient and contraction amplitudes in individual cardiac ventricular cells, are, in part, due to a reduction in  $I_{Ca}$ .

Combined into Z01 AG 00271-03 LCS.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00261-05 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Secondary  $[Ca^{2+}]_i$ -Dependent Modulation of Contractility in Single Cardiac Myocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. J. Sollott	Senior Staff Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	H. A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

It is well established that the primary modes of altering myocardial contractility on a beat-to-beat basis involve both the modulation of the myoplasmic  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) availability at the level of the myofilaments (MF), and changes in the  $Ca^{2+}$  response of the MF, especially via length-dependent activation. Alternatively, certain more slowly developing covalent modifications of MF have been recognized (such as thick or thin filament phosphorylation), but these are of uncertain functional significance in cardiac tissue. We have found that the  $[Ca^{2+}]_i$  "history" regulates contractility via myosin light chain (MLC<sub>2</sub>) phosphorylation, based on the results of  $[Ca^{2+}]_i$ -clamp experiments at various levels (ranging up to 10-fold > resting  $[Ca^{2+}]_i$ ) and durations (up to 60s) via 10 Hz tetanization in sarcoplasmic reticulum-disabled (thapsigargin or caffeine treated) intact rat cardiac myocytes loaded with indo-1 free acid. The rising phase of the tetanus (2-3 sec) was sufficiently slow to permit continuous equilibration of  $Ca^{2+}$ -MF binding, and thus force and cell length, enabling a rapid assessment of baseline contractile activation. Secondary  $[Ca^{2+}]_i$ - and time-dependent progressive cell shortening was observed despite steady levels of clamped  $[Ca^{2+}]_i$ , representing a progressive leftward shift in the length-pCa curve (without changing  $F_{max}$  nor the Hill coefficient) due to MLC<sub>2</sub> phosphorylation. The effect of protein phosphatase inhibitor calyculin A, which (nonspecifically) increases MLC<sub>2</sub> phosphorylation and left-shifts the L-pCa curve (without changing  $F_{max}$ ) was rapidly reversed by the nonspecific phosphatase 2,3-butanedione monoxime (BDM), which reduces MLC<sub>2</sub> phosphorylation and  $F_{max}$ , and shifts the L-pCa curve to the right. We propose that  $[Ca^{2+}]_i$  history, via  $Ca^{2+}$ -calmodulin-dependent MLCK activity, myosin light chain phosphorylation, and kinase/phosphatase balance, plays a significant modulatory role in the chronic regulation of contractility in intact heart cells. This integrative phenomenon would reflect a history dependence of cardiac work/demand, and may serve as an important cardiovascular adaptive mechanism in myocardial conditioning.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00263-04 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism Affects Calcium in Cultured Aortic Endothelial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. Silverman	Guest Researcher	LCS, NIA
Others:	R. Ziegelstein	Clinical Associate	LCS, NIA
	L. Cheng	Research Chemist	LCS, NIA
	M. Stern	Guest Researcher	LCS, NIA
	E. Lakatta	Chief	LCS, NIA

COOPERATING UNITS (if any)

Johns Hopkins University, Division of Cardiology (H. Silverman, M. Stern)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hypoxia is known to affect both endothelial cell adenine nucleotide metabolism and release of vasoactive substances, but the link between these is unknown. Since the release of these vasoactive substances may be in part mediated by an increase in  $[Ca^{2+}]_i$ , we investigated the effects of metabolic inhibitors on  $[Ca^{2+}]_i$  in indo-1 loaded cultured rat aortic endothelial cells. Inhibition of oxidative phosphorylation (NaCN, 2 mM) or substrate deprivation alone (no glucose) caused little change in  $[Ca^{2+}]_i$ . In contrast, combined inhibition of oxidative phosphorylation and substrate deprivation (NaCN, no glucose) caused a significant increase in  $[Ca^{2+}]_i$ .  $[Ca^{2+}]_i$  rose similarly even when cells were studied in the absence of external  $Ca^{2+}$ . The greatest increase in  $[Ca^{2+}]_i$  occurred in the absence of substrate and during exposure to an inhibitor of glycolysis (iodoacetate, 2 mM). These results suggest a lack of endogenous oxidative substrate and a critical dependence on glycolytic metabolism.

Combined into Z01 AG 00226-11 LCS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00264-04 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Age, Gender and  $\beta$  Blockade on Resting and Exercise Cardiac Performance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. Lakatta Chief LCS, NIA  
Others: J. Fleg Unit Chief LCS, NIA  
F. O'Connor Statistician (Health) LCS, NIA

COOPERATING UNITS (if any)

Division of Cardiology, Johns Hopkins Hospital (G. Gerstenblith, S. Schulman, L. Becker)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

6.5

PROFESSIONAL:

2.5

OTHER:

4.0

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. Although the effects of age on the cardiac response to maximal aerobic exercise are well known in men, whether women undergo similar aging changes is unknown. To determine the independent effects of age and gender on the left ventricular response to exercise, we performed gating blood pool scans at rest and maximal upright cycle exercise in 121 men and 79 women ages 22-86 yrs free of heart disease by history, physical exam, rest and exercise ECG and if > 40 yr old, exercise thallium scan. Maximal cycle workload declined similarly with age in men (36%) and women (42%) between the third and ninth decades, although the absolute maximal load achieved was higher in men for any given age. At seated rest age-associated declines in heart rate (HR) and increases in systolic blood pressure (SBP) were observed in both sexes. Resting end-diastolic volume index (EDVI) and stroke volume index (SVI) rose with age in men but not in women. In both sexes, maximal heart rate, ejection fraction and cardiac index declined with age whereas end systolic volume index (ESVI) and total systemic vascular resistance (TSVR) increased. Although EDVI at maximal effort increased with age in men but not in women, SVI was not age related in either sex. Thus, aging and gender have distinct influences on the cardiac response to maximal cycle exercise. B. The response to strenuous aerobic exercise (EX) is mediated in large part by beta-adrenergic activation, the efficiency of which declines with advancing age. To ascertain the importance of the beta-adrenergic system on age-associated changes in hemodynamic during EX, we performed maximal cycle EX in 25 healthy men ages 28-72 yr from the BLSA after acute beta blockade with intravenous propranolol. In these men, EDVI at peak workload declined with age ( $r = -0.45$ ) causing an age-associated decline in SVI ( $r = 0.48$ ,  $p < 0.05$ ) not present in 70 unblocked men. The decline in HR with age in propranolol-treated men was blunted ( $0.46$  beats/min/yr) compared to controls ( $1.09$  beats/min/yr). Maximal LVEF declined with age similarly with ( $r = -0.50$ ,  $p < 0.01$ ) and without ( $r = -0.45$ ,  $p < 0.0001$ ) beta-adrenergic blockade. The primary reason for the slope shifts in the age regressions propranolol was a large increase in EDVI and SVI and a large decrease in HR in younger men. Similarly, IV propranolol resulted in reduced peak filling rates with EX in young ( $27 \pm 8$  yr) but not older ( $62 \pm 6$  yr) men relative to their unmedicated age peers. We conclude that age differences in beta-adrenergic responsiveness underlie many of the age-associated changes in hemodynamic during vigorous aerobic EX.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00265-04 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Age and Gender on Cardiac Structure and Exercise Cardiac Performance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Fleg Staff Cardiologist LCS, NIA  
Others: F. O'Connor Chemist LCS, NIA  
E. Lakatta Chief LCS, NIA

COOPERATING UNITS (if any)

Division of Cardiology, Johns Hopkins Hospital (G. Gerstenblith, J. Lima, S. Schulman, L. Becker, V. Coombs, J. Clulow).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4

PROFESSIONAL:

1.5

OTHER:

2.5

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

To determine the independent effects of age and gender on the left ventricular structure and response to upright cycle exercise, we analyzed resting echocardiograms and gated blood pool scans at rest and maximal upright cycle exercise in 95 men and 50 women ages 23 - 86 yrs free of heart disease by history, physical exam, rest and exercise ECG and if > 40 yr old, exercise thallium scan. Left ventricular end-diastolic wall thickness increased with age ( $r = 0.42$ ) and with body surface area ( $r = 0.38$ ) in both sexes; after normalization for body size, there was no gender difference in the age-wall thickness relationship. Maximal cycle workload declined similarly with age in men (41%) and women (47%) between the third and ninth decades, although the absolute maximal load achieved was higher in men for any given age. At seated rest age-associated declines in heart rate (HR) and increases in systolic blood pressure (SBP) were observed in both sexes. Resting end diastolic volume index (EDVI) and stroke volume index (SVI) rose with age in men but not in women. At a fixed external workload of 50 watts, heart rate decreased and EDVI, ESVI, and SVI increased with age in men but not in women. In both sexes, maximal heart rate, ejection fraction and cardiac index declined with age whereas end systolic volume index (ESVI) and total systemic vascular resistance (TSVR) increased. Although EDVI at maximal effort increased with age in men but not in women, SVI was not age related in either sex. When men and women younger than 40 years who achieved similar maximal workloads of 125-150 watts were compared across workloads, women had higher heart rates and thence higher cardiac indices than their male counterparts whereas the men had higher systolic blood pressure responses. Fitness-matched older (> 60 years) men and women demonstrated similar exercise hemodynamics except for a higher heart rate response in the latter. Thus, gender has a significant influence on the age-associated changes in resting and exercise hemodynamics in normal humans. Older men appear to utilize the Frank-Starling mechanism to augment cardiac output more than older women, whereas the latter have a higher heart rate response. However, the apparent gender differences in left ventricular wall thickness are eliminated by normalization for body size.

Combined into Z01 AG 00264-04 LCS.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00266-04 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ion Transport Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. P. Froehlich Chief, MBS LCS, NIA

Others: J. L. Kinsella Research Physiologist LCS, NIA  
R. W. Albers Chief, Enzymes Section LNC, NINCDS  
P. F. Heller Chemist LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD; Max-Planck-Institute for Biophysics, Frankfurt, Germany (K. Fendler); Department Biochemistry, U. of Minnesota (D. Thomas and J. Mahaney).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.1

PROFESSIONAL:

2.1

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

In eukaryotic cells, transmembrane gradients for  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  are established and maintained by ATP-dependent ion pumps. Deterioration of these systems with age can lead to alterations in cellular ion homeostasis and loss of cell function. This study focuses on the basic transport mechanisms utilized by ion pumps to gain insight into the molecular basis of altered transport function during aging.

The biochemical expression of the plasma membrane Na,K pump is an ATPase which cycles through phosphorylated and dephosphorylated intermediate states. The kinetic behavior of the ATPase partial reactions is complex and cannot be explained by a simple consecutive mechanism. Recent rapid mixing experiments have shown that some of these complex effects can be eliminated by treatment with non-solubilizing concentrations of n-dodecyl  $\beta$ , D maltoside, a non-ionic detergent. We propose that interactions between  $\alpha$  catalytic subunits are responsible for the complex behavior and that the detergent disrupts hydrophobic subunit-subunit contacts which are necessary for optimal pumping rates and energy utilization.

Rapid mixing and time-resolved EPR studies of the  $\text{Ca}^{2+}$  pump in sarcoplasmic reticulum have produced evidence for a substrate-activated conformational state which participates in  $\text{Ca}^{2+}$  translocation. Relaxation of this conformational state is associated with a large free energy change resulting in  $\text{Ca}^{2+}$  release into an occluded compartment near the inner membrane surface. The kinetic behavior of the Ca-ATPase is compatible with a dimer in which energy-yielding and energy-requiring reactions in adjacent subunits are coupled to optimize  $\text{Ca}^{2+}$  pumping.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00267-04 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Cell Function and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.I. Cheng	Research Chemist	LCS, NIA
	J.P. Froehlich	Chief, MBS	LCS, NIA
Others:	E. Koh	Visiting Fellow	LCS, NIA
	E.G. Lakatta	Chief, LCS	LCS, NIA
	J.L. Kinsella	Research Physiologist	LCS, NIA
	C.T. Liang	Research Chemist	LBC, NIA

COOPERATING UNITS (if any)

Laboratory of Biological Chemistry, NIA (C.T. Liang)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.45

PROFESSIONAL:

2.45

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A. Aging constitutes a risk factor in the development of cardiovascular disease. Older animals are more susceptible to vascular lesions after endothelial injury than are young animals. The mechanism leading to the increased proliferation of SMC in the intima is unknown. However, the intimal thickening may result from excessive production of growth factors and/or increasing the activities of proteinases known to enhance the degradation of the extracellular matrix components. The purpose of these studies was to examine the factors involved in the development of intimal thickening. Histological staining of the thoracic aorta with TGF- $\beta$  specific antibodies have revealed the presence of higher levels of extracellular TGF $\beta$ , in the basement membranes from old rats as compared to young. In contrast, no apparent age-related differences were detected in the levels of intracellular TGF $\beta$ , or extracellular TGF $\beta$ , and TGF $\beta$ . Significant elevations in collagenase activity were detected in aortic homogenates from old and balloon injured animals. These elevations may be involved in the degradation of matrix protein associated with SMC migration and intimal thickening.

B. Age-related alterations in smooth muscle ion metabolism may contribute to the onset and progression of hypertension. A model for the investigation of this process involves freshly-isolated single rat arterial smooth muscle cells (SMC). These cells were used to investigate the relationship between intracellular  $[Ca^{2+}]$  fluctuations and initial velocity of cell shortening induced by norepinephrine and  $K^+$  depolarization. The results showed that single cells retain a  $Ca^{2+}$ -dependent variation in shortening velocity consistent with the behavior observed in multicellular (tissue) preparations.

Combined into Z01 AG 00817-01 LCS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00269-04 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

$\alpha$ -Adrenergic Stimulation in Myocardial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Gambassi Visiting Fellow (DOD 2/20/93) LCS, NIA  
Others: H. A. Spurgeon Physiologist LCS, NIA  
E. G. Lakatta Chief LCS, NIA  
M. C. Capogrossi Medical Officer LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously shown that the positive inotropic action of  $\alpha_1$ -adrenoceptor ( $\alpha_1$ -AR) stimulation, at least in part is due to an enhanced myofilament responsiveness to  $Ca^{2+}$  mediated by protein kinase C (PKC)-dependent activation of  $Na^+/H^+$  exchange and an increase in cytosolic pH (pH<sub>i</sub>). We have also examined the effect of  $\alpha_1$ -AR subtypes,  $\alpha_{1A}$  and  $\alpha_{1B}$  on contraction,  $Ca_i$  and myofilament response to  $Ca^{2+}$  of isolated myocardial cells.  $\alpha_{1A}$ -AR stimulation (phenylephrine, nadolol and  $\alpha_{1B}$ -AR inactivation with chloroethylclonidine) increased contraction,  $Ca_i$  transient amplitude and myofilament response to  $Ca^{2+}$ . In contrast  $\alpha_{1B}$ -AR stimulation (phenylephrine, nadolol and  $\alpha_{1A}$ -blockade with WB-4101) decreased contraction,  $Ca_i$  transient amplitude and downregulated the  $\alpha_{1A}$ -AR effect to increase myofilament response to  $Ca^{2+}$ . In additional experiments we have examined the effect of  $\alpha_{1A}$ - and  $\alpha_{1B}$ -AR stimulation on pH<sub>i</sub> and the PKC-dependence of the  $\alpha_{1B}$ -AR effect on pH<sub>i</sub> and contraction of isolated myocardial cells. In  $HCO_3^-/CO_2$ -buffered saline,  $\alpha_{1A}$  increased cytosolic pH. In contrast  $\alpha_{1B}$  decreased cytosolic pH and this effect persisted in  $HCO_3^-/CO_2$ -free solution.  $\alpha_{1B}$ -mediated cytosolic acidification was abolished by staurosporine, a protein kinase C inhibitor, and by protein kinase C down-regulation with prolonged exposure to 4 $\beta$ -phorbol 12-myristate 13-acetate. Changes in twitch amplitude paralleled those in cytosolic pH due either to  $\alpha_{1A}$ - or  $\alpha_{1B}$ - adrenoceptor stimulation. Our results show that  $\alpha_{1A}$ -AR and  $\alpha_{1B}$ -AR have opposite effects on pH<sub>i</sub> homeostasis of isolated myocardial cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00270-04 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age-Associated Changes in Vascular Stiffness Properties

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Lakatta	Chief	LCS, NIA
Others:	P. Vaitkevicius	Guest Researcher	LCS, NIA
	J. Fleg	Unit Chief	LCS, NIA
	J. Engel	Computer Specialist	RRB, NIA
	H. Spurgeon	Physiologist	LCS, NIA

COOPERATING UNITS (if any)

Johns Hopkins University (E. Shapiro, G. Gerstenblith)  
University of Maryland (A. Goldberg, L. Lakatta)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In industrialized societies increases in arterial stiffness and left ventricular (LV) mass are considered part of normative aging. However, considerable heterogeneity exists in the age-associated changes in these variables both across cultures as well as within a given population. The ultimate goals of this project are to address the issue of how alterations in arterial stiffness affect the myocardium and whether in outcome studies they relate to vascular insufficiency syndromes, e.g., stroke. We have initiated a pilot study in which measurements of cardiac mass (via NMR), filling properties and isovolumic relaxation time (via Doppler echocardiography), carotid pressure pulse (via applanation tomography) and arterial pulse wave velocity (Doppler sonography) are made in men and women who differ with respect to age, race, arterial pressure, body composition, and physical conditioning status. As expected, results indicate that with increasing age arterial stiffening leads to an increase in pulse wave velocity. This is associated with an early return of reflected pulse waves from peripheral sites which produce an augmented and late occurring peak of carotid pressure pulse. It is important to note that these arterial changes are, by and large, not detected by routine clinical measures of brachial arterial pressure and are independent of gender, but vary inversely with exercise capacity. Of note, also is that in highly physically conditioned older individuals (>60 yrs of age) the arterial stiffness and reflected wave indices are markedly reduced relative to their sedentary age peers and do not differ from those of younger individuals, although the age-associated increase in systolic pressure persists. We have undertaken an initiative to extend many of these studies to Black Americans and to other non-Western populations in which different patterns of arterial pressure change occurs with aging (in China via a research contract (cf. #N01-AG-02-2118)). Long term goals include determination of whether "normative" age-associated changes in cardiac structure (i.e. increasing left ventricular mass) and function (delayed left ventricular relaxation and reduced early diastolic filling rates) are directly related to changes in arterial stiffness. If such is the case, a long-term intervention trial might be justified to see whether amelioration of arterial stiffness by medication or lifestyle intervention can attenuate these age-associated changes in cardiac structure and function and reduce future cardiovascular morbidity.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00271-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Involvement of Cardiac Opioids in Response of the Heart to Stress

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Boluyt NRC Fellow LCS, NIA

Others: E.G. Lakatta Chief LCS, NIA  
M. Crow Senior Staff Fellow LCS, NIA  
L. O'Neill Biologist LCS, NIA  
J.S. Zheng Staff Fellow LCS, NIA

COOPERATING UNITS (if any)

Department de Biologie Appliquee, Universite d'Auvergne Clermont, Aubiere, France (A. Younes); Texas College of Osteopathic Medicine, Forth Worth, Texas, (J. Faffrey)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous work in the LCS has shown that opioid peptides can directly modulate cardiac myocyte contractile function and inhibit catecholamine induced inotropy by affecting signal transduction through the  $\beta$ -adrenergic pathway. Since both contractile force and  $\beta$ -adrenergic mediated events are involved in the hypertrophic process, we sought to determine whether the cardiac response to hypertrophic stress is associated with changes in opioid peptide levels in rat heart, whether opioid peptide production is regulated at the level of gene expression, and investigated factors that regulate opioid gene expression in the heart. We studied opioid peptide production and/or proenkephalin (PNK) gene expression in 3 well established models of cardiac hypertrophy: aging, aortic constriction and  $\alpha$ -adrenergic stimulation in culture. Cardiac opioid peptide was upregulated 2-3-fold with advancing age, and PNK gene expression was elevated in the heart during aging (7-fold), and during chronic pressure overload hypertrophy leading to heart failure (2-fold). Paradoxically, PNK gene expression was transiently downregulated 3 days after aortic constriction, and this transient decrease was abolished by prior chemical sympathectomy. Norepinephrine (NE) treatment increased the level of PNK mRNA in myocyte cultures (peak 4 hr). Neither  $\alpha$ - nor  $\beta$ -adrenergic receptor stimulation alone was sufficient to produce PNK induction. The induction of PNK by NE in myocytes did not occur unless myocytes were co-cultured with cardiac fibroblasts (in situ hybridization), and could not be reproduced with conditioned media from cardiac fibroblasts. The induction of hypertrophy marker genes (ANF and  $\alpha$ -skeletal actin) by NE in myocyte cultures was not diminished by adding opioid peptides to the media, nor augmented by supplementing the media with opioid receptor antagonists. In summary, opioid peptide production is augmented during chronic cardiac hypertrophy, and is regulated, in part, at the level of gene expression. The negative modulatory influence of opioid peptides on cardiac myocytes may be limited to functional parameters, and apparently does not affect hypertrophic growth of myocytes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00272-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms for Signal Transduction of Shear Stress Forces in Endothelial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Ziegelstein	Guest Researcher	LCS, NIA
Others:	M. Capogrossi	Medical Officer	LCS, NIA
	L. Cheng	Research Chemist, MBS	LCS, NIA
	G. Zheng	Special Volunteer	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The vascular endothelium, positioned between the flowing blood and the vessel wall, is uniquely exposed to hemodynamic shear stress forces. To study the effect of shear stress forces on vascular endothelial pH and cytosolic  $[Ca^{2+}]_i$  ( $[Ca^{2+}]_i$ ), cells were cultured in 1 mm<sup>2</sup> glass capillary tubes, loaded with the fluorescent indicator carboxy-seminaphtharhodafleur-1 (SNARF-1 for pH<sub>i</sub>) or indo-1 ( $[Ca^{2+}]_i$ ) and studied on the stage of a modified inverted fluorescence microscope. These capillary tubes facilitate pH<sub>i</sub> or  $[Ca^{2+}]_i$  measurements in a closed system which does not allow gas diffusion when using CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>-buffered solutions; small changes in flow rate result in relatively large changes in shear stress forces. We have recently reported that flow-dependent intracellular acidification occurs in endothelial cells during brief exposures to continuous laminar shear stress forces in a physiologic buffer with bicarbonate due to parallel activation of Na<sup>+</sup>-independent Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange and Na<sup>+</sup>/H<sup>+</sup> exchange (Science 258: 656-659, 1992). This change in intracellular pH (pH<sub>i</sub>) is sustained during a 30 minute exposure to shear stress forces of 13.4 dyne cm<sup>-2</sup>, although partial recovery from the acidification occurs during a 30 minute exposure to shear stress forces of 2.7 dyne cm<sup>-2</sup> or less. To determine the mechanism of the partial recovery of pH<sub>i</sub> during a 30 minute exposure to shear stress forces of 2.7 dyne cm<sup>-2</sup> or less, cells were exposed to ethylisopropylamiloride (EIPA), a Na<sup>+</sup>/H<sup>+</sup> exchange inhibitor or to Na<sup>+</sup>-free buffer to inhibit Na<sup>+</sup>-dependent exchange mechanisms. While EIPA had no effect, removal of buffer Na<sup>+</sup> significantly inhibited the pH<sub>i</sub> recovery. These results suggest that while Na<sup>+</sup>-dependent Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange is also activated by hemodynamic shear stress exposure. Studies were also performed to characterize the pH<sub>i</sub> response following a 30 minute exposure to shear stress forces. After return to control conditions, a slowly-developing increase in endothelial pH<sub>i</sub> of approximately 0.20 pH units has been noted on return to control conditions. Following this alkalization, pH<sub>i</sub> recovers to control values over 15-20 minutes. Thus, pH<sub>i</sub> appears to play a significant role in the response of the vascular endothelium to shear stress forces.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00273-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

MyoD1 and Developmental Gene Expression in Skeletal Muscle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Crow Senior Staff Fellow LCS, NIA

Others: N. Papadopoulos Visiting Fellow LCS, NIA

COOPERATING UNITS (if any)

Baylor College of Medicine, Houston, Texas (Robert Schwartz); University of Texas, Houston, Texas (Eric N. Olson)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The skeletal muscle regulatory factors (MRFs), of which myoD is the prototype, are a family of helix-loop-helix proteins that act as transcription factors for skeletal muscle-specific gene expression. The various members of the MRF family are differentially expressed during development suggesting that they may participate in orchestrating developmental changes in skeletal muscle maturation. To address this, we have focussed on the changes in MRF gene expression that occur between embryonic and fetal stages of chicken skeletal muscle development. This transition involves the switching of contractile protein isoform gene expression from the "cardiac" to the "skeletal" type, occurs in concert with increases in muscle activity, and is associated with increased protein kinase C (PKC) activity. In work completed this year, we show that expression of the MRFs and "cardiac" type contractile proteins mRNA in cultured muscle cells are decreased when PKC levels are elevated either pharmacologically or genetically. We also show that the effect of PKC on myoD is at the level of gene transcription, but cannot be overcome by overexpression of myoD driven by a heterologous promoter. Since MRFs are likely to positively regulate their own gene expression, the data is consistent with a model of regulation in which the primary effect of PKC is post-transcriptional, creating a defunct transcription factor that can neither transactivate its own gene or that of its targets. Additional information suggests that the effect is likely mediated by direct phosphorylation of a threonine residue in the DNA binding domain of the MRFs.

Combined into Z01 AG 00274-03 LCS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00274-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Smooth Muscle Gene Expression and Cellular Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Crow	Senior Staff Fellow	LCS, NIA
Others:	N. Papadopoulos	Visiting Fellow (DOD 3/1/93)	LCS, NIA
	C. Bilato	Guest Researcher	LCS, NIA
	J. Fredman	IRTA	LCS, NIA
	M. Chin	IRTA	LCS, NIA
	R-P. Xiao	Visiting Associate	LCS, NIA
	R. Pauly	Senior Staff Fellow	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The phenotypic modulation of vascular smooth muscle cells (VSMCs) from a quiescent, differentiated state to that of proliferating, "undifferentiated" cells is a common pathogenic feature of vascular disease. The molecular mechanisms by which this phenotypic "switch" is achieved are unknown. We have observed that proliferating vascular smooth muscle cells express mRNAs that are homologous to a gene expressed in other tissues that acts as a transdominant suppressor of differentiation. This gene is referred to as ID (inhibitor of differentiation). It belongs to the family of helix-loop-helix (HLH) family of proteins, many of which are involved in cell determination and differentiation. Using a variety of molecular cloning techniques, we have further identified and cloned two ID-like cDNAs from proliferating VSMCs. One of these is the rat homologue of mouse ID1 (i.e. rat ID1) and the other is apparently a unique member of the ID family. We have further demonstrated that rat ID1 exists as two protein isoforms which are the products of alternative RNA splicing of the rat ID1 gene. Expression of rat ID1 is upregulated by a number of growth factors that are important in initiating and sustaining VSMC proliferation, such as PDGF, bFGF, and IGF-1. Expression of rat ID1 is often (but not always) downregulated when VSMCs are made quiescent. However, when cultured VSMCs achieve a level of differentiation comparable to that in the intact vessel (for example, when they are cultured on reconstituted basement membrane), ID expression decreases 10-fold to a level similar to that seen in the intact, uninjured vessel. Our group is currently initiating studies to identify the function of ID in VSMC migration, proliferation, and differentiation by overexpressing rat ID1 from constitutively active and inducible promoters, by antisense inhibition of rat ID1, and by genetic screening of cDNA libraries using the two hybrid yeast system to identify potential protein partners for rat ID1.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00275-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modulation of Myofilament  $Ca^{2+}$  Sensitivity as a Positive Inotropic Intervention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.G. Lakatta	Chief	LCS, NIA
Others:	G. Gambassi	Visiting Fellow	LCS, NIA
	M.C. Capogrossi	Medical Officer	LCS, NIA
	H.A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

E. Merck, Darmstadt, Germany (M. Klockow)  
Dept. of Physiology, University of Chicago at Illinois (J.R. Solaro)  
Dept. of Physiology, University of Vermont (D. Warshaw)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2

OTHER:

0.5

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A persistent challenge has been the development of substances that increase myocardial contractility via an enhancement of myofilament responsiveness to  $Ca^{2+}$  rather than by increasing the extent of cellular  $Ca^{2+}$  loading. The diazinone derivative, EMD 53998 (designed by E. Merck, Darmstadt, Germany), increases the peak force and shift leftward the  $pCa$ -force relationship in skinned myocardial fibers; and in cell homogenates it exhibits phosphodiesterase (PDE) inhibitory activity. However, the potency of myofilament sensitization relative to that of PDE inhibition is greater than for any known substance. The effects of EMD 53998, and of its (+), EMD 57033 and (-), EMD 57439, enantiomers, were tested on the contractile properties and  $Ca$  transients of single, intact, guinea pig and dog cardiac myocytes. Cells were loaded with the fluorescent dye, indo-1, and bathed in a Hepes buffer at 25°C. Our aim was to ascertain whether the optical enantiomers could separate the effect mediated through PDE inhibition from that obtained via an increased myofilament responsiveness to  $Ca^{2+}$ . All three substances exerted a pronounced increase in twitch amplitude: the maximal effect of the racemate was approximately the sum of the effects of its two enantiomers. The  $Ca$  transient, measured as the 410/490 nm indo-1 fluorescence ratio transient, was increased by the racemate and its (-) enantiomer, but not by the (+)-enantiomer. In unstimulated cells resting length was significantly reduced by the (+)-enantiomer and this was accompanied by a decrease in indo-1 fluorescence; the (-)-enantiomer had no effect on either parameter. Qualitatively similar effects were obtained in experiments with intact dog cardiac cells. The molecular mechanism of the effect of the (+)-enantiomer was further studied in dog cardiac myofibrils. EMD 57033 stimulates the ATPase activity in myofibrils in which troponin-tropomyosin have been extracted, but does not affect  $Ca^{2+}$  binding to isolated troponin C. Furthermore, in a motility assay containing isolated actin and myosin, but devoid of  $Ca^{2+}$  and regulatory proteins, the substance increases the velocity of actin motion along myosin. Thus, in intact cells the (+)-enantiomer of EMD 53998 has direct myofilament effects which are not simply due to " $Ca^{2+}$ -sensitization" (i.e. effects downstream to  $Ca^{2+}$ -binding to troponin C and regulatory protein modulation of thin filament activation); rather an effect on acto-myosin interaction, i.e. the cross-bridge is likely involved. (Combined into Z01 AG 00261-05 LCS).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00276-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mitochondrial and Cytosolic Calcium in Cardiac Myocyte After Anoxia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. Silverman	Guest Researcher	LCS, NIA
Others:	H. Miyata	Visiting Fellow (DOD 2/25/92)	LCS, NIA
	E. Lakatta	Chief	LCS, NIA
	M. Stern	Guest Researcher	LCS, NIA

COOPERATING UNITS (If any)

Johns Hopkins University, Division of Cardiology (H. Silverman, M. Stern)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies from this laboratory and others have documented an association between cellular  $\text{Ca}^{2+}$  loading and cardiac myocyte death following exposure to anoxia. It remains unclear whether these changes in  $\text{Ca}^{2+}$  mediate cell injury and how this may occur. We characterized the changes in  $\text{Ca}^{2+}$  which occurred in the cytosolic compartment  $[\text{Ca}^{2+}]_i$  and mitochondrial compartment  $[\text{Ca}^{2+}]_m$  of adult rat cardiac myocytes exposed to hypoxia/reoxygenation to define the relation between subcellular  $\text{Ca}^{2+}$  regulation and cell injury. During anoxia,  $[\text{Ca}^{2+}]_i$  and  $[\text{Ca}^{2+}]_m$  rose only after the onset of ATP-depletion rigor contracture. At reoxygenation 35 minutes later,  $[\text{Ca}^{2+}]_i$  fell rapidly to preanoxic levels while  $[\text{Ca}^{2+}]_m$  fell more slowly. Cellular hypercontracture or death was associated with a rise in  $[\text{Ca}^{2+}]_i$  or  $[\text{Ca}^{2+}]_m$  to greater than 250 nM just prior to reoxygenation. Reversible relatively minor rises in  $[\text{Ca}^{2+}]_m$  may be seen and do not predict cell death. The cause of cell death remains unclear but does not appear to be the result of massive reoxygenation-induced  $\text{Ca}^{2+}$  overload.

Combined into Z01 AG 00206-11 LCS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00277-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Angiogenesis Endothelial Cell Function and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. Kinsella	Research Physiologist	LCS, NIA
Others:	L. Cheng	Research Chemist	LCS, NIA
	J. Froehlich	Chief, MBS	LCS, NIA
	E. Lakatta	Chief	LCS, NIA
	M. Kuzuya	Visiting Fellow (DOD 7/1/93)	LCS, NIA
	P. Heller	Chemist	LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Developmental Biology, NIDR, NIH (H. Kleinman, D. Grant and B. Weeks) and Laboratory Clinical Physiology NIA NIH (J. Chrestl).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.7

PROFESSIONAL:

1.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Angiogenesis (the formation of new blood vessels) is an important process in tumor growth, wound healing, retinopathies, and rheumatoid arthritis. We are investigating aspects of angiogenesis in an in vitro model utilizing isolated human and bovine endothelial cells and a mixture of basement membrane matrix proteins (Matrigel) or collagen gel.

Normally endothelial cells are quiescent in vivo; but in response to an angiogenic factor, activated endothelial cells will breakdown their underlying basement membrane, migrate into the interstitium, proliferate, and finally differentiate into a new blood vessel. We found that quiescent endothelial cells (contact inhibition or serum starvation) were not able to organize into a capillary-like network in vitro, while cells released from contact inhibition or feed serum-containing media regained the ability to organize. There was a correlation between the ability to organize on Matrigel and the expression of cell surface receptors for the basement membrane proteins, laminin and collagen IV. These studies should prove useful in studying some early events involved in angiogenesis.

Angiogenic factors attract leukocytes and fibroblast in an area, while stimulating the formation of new blood vessels. We tested whether fibroblast produced some factor that altered the ability of endothelial cells to organize into new vessels. We found that fibroblast-conditioned media contained a cellular attractant and enhanced the extent of capillary-like formations on Matrigel or within a collagen gel. The ability of fibroblast to influence angiogenesis may be an important component of wound-healing.

We found that TGF- $\beta$  caused differentiated endothelial cells on Matrigel or on collagen 1 gels to undergo apoptosis. TGF- $\beta$  may be important for the final stages of wound healing when the area forms scar tissue and becomes avascular.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00278-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Vascular Smooth Muscle Cells in Vascular Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Pauly	Senior Staff Fellow	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	R. Monticone	Biologist	LCS, NIA
	Y. Gluzband	Chemist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The migration, proliferation, and neointimal accumulation of vascular smooth muscle cells (VSMCs) are key events in the development and progression of many vascular diseases and a predictable consequence of mechanical injury to the blood vessel. VSMCs in vivo are surrounded by and embedded in extracellular matrices (ECMs) that must be traversed during migration. In many other cell types, migration across ECM barriers involves the local destruction or degradation of these barriers by extracellular proteases. Principle among such proteases are those belonging to the matrix metalloproteinase (MMP) family. Using an in vitro assay to monitor and manipulate the ability of VSMCs to degrade a defined ECM barrier as they migrate toward a chemoattractant, we demonstrate the VSMCs isolated from the rat thoracic aorta and maintained in a proliferating or "synthetic" state readily migrate through an ECM barrier of reconstituted basement membrane. The migration of serum-starved/differentiated VSMCs toward the chemoattractant both in the presence and in the absence of the barrier is less than 20% ( $p < 0.001$ ) that of proliferating cells. The importance of MMP expression during the migration of "synthetic" VSMCs through the reconstituted BM was demonstrated using a peptide that mimics the inhibitory propeptide region of all MMPs. This peptide blocked migration of proliferating cells through the barrier by more than 80% ( $p < 0.005$ ), but did not significantly affect migration that occurred in the absence of the barrier. Likewise, antisera capable of neutralizing the activity of the 72 kD type IV collagenase (MMP-2) also inhibited migration through the barrier, without significantly affecting the migration of cells in the absence of the barrier. Northern blotting and zymographic analyses indicate that MMP2 is the principal MMP expressed and secreted by these cells. MMP2 activity expressed by serum starved/differentiated VSMCs as measured by a fluorescent peptide cleavage assay was less than 5% of that measured in proliferating VSMCs. These results demonstrate that VSMCs migrate through an ECM barrier similar in composition to one that normally surrounds them and that this ability is regulated by the phenotypic state of the cell.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00279-03 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Comparison  $\beta_1$  vs  $\beta_2$  Adrenoceptor Stimulation in Rat Cardiocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R-P. Xiao Visiting Associate LCS, NIA

Others: E.G. Lakatta Chief LCS, NIA

COOPERATING UNITS (if any)

Department of Medical Biochemistry, Ohio State University (Ruth Altschuld, Ph.D.);  
Department of Biochemistry, Indiana University School of Medicine (Larry Jones,  
Ph.D.)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

$\beta$ -adrenergic receptor ( $\beta$ AR) stimulation has profound modulatory effects on the cardiac contraction. It has been well documented that both the  $\beta_1$  and  $\beta_2$  AR subtypes are coupled to adenylylase and that their stimulation by  $\beta_1$  and  $\beta_2$  AR specific agonist leads to an increase in cAMP. Stimulation of other heart cell receptors, e.g. prostaglandin, also leads to an increase in cAMP but has no effect on contraction, presumably because the cAMP pool affected is not associated with membranous cAMP activation and is not linked to heart cell  $Ca^{2+}$  regulation. It is widely recognized that stimulation of  $\beta$  AR's leads to an increase in the particulate (membrane bound) cAMP levels and protein kinase (PK) phosphorylation of key proteins involved in excitation-contraction coupling. However, whether  $\beta$  AR stimulation increases particulate cAMP and cAMP dependent phosphorylation is not known. In this regard we have recently shown that the  $\beta_2$  AR mediated effects on  $Ca^{2+}$ , contraction and L type  $Ca^{2+}$  channels in rat heart cells differ markedly from those elicited by  $\beta_1$  AR stimulation. In this project we further demonstrated that the  $\beta_2$  effects on  $Ca^{2+}$  and contraction in these cells are not mediated by cAMP. This conclusion is based on the measurement of total and particulate cAMP levels and the decoupling between the increase of cAMP levels and increases in cell contraction and  $Ca^{2+}$  transient. Furthermore, phosphorylation of sarcoplasmic reticulum (SR) phospholamban was dramatically increased by  $\beta_1$  AR stimulation, but not by  $\beta_2$  AR stimulation. Thus,  $\beta_1$  and  $\beta_2$  AR are coupled to cellular effects of altered  $Ca^{2+}$  homeostasis and contraction via different signal transduction pathways.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00800-02 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Energy Metabolism in Aging and Disease: Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. G. Hansford Chief, EMBS LCS, NIA

Others: B. Hogue Chemist LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Biological Chemistry (Dr. C.F. Filburn)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Energy Metabolism and Bioenergetics Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.4

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise survival of neurones. We have investigated the frequency of occurrence of a major deletion (4.8 Kb) in mitochondrial (mt) DNA in four defined brain regions (cerebral cortex, cerebellum, hippocampus and striatum), as a function of aging in the rat. mt-DNA codes for subunits of complexes I, III and IV of the mitochondrial respiratory chain as well as complex V (the ATP-synthase), ribosomal and t-RNA's. The magnitude of the major deletion which we have studied is such as to make transcription completely incompetent for the affected DNA molecule; however, complementation with other DNA molecules within the same mitochondrion is likely to occur. We found increases with aging of approximately 6, 10 and 20-fold in the incidence of the 4.8 Kb deletion, as a fraction of total genomes, when cerebral cortex, hippocampus and striatum, respectively, were compared in 6 month and 22-23 month old rats. However, the deleted genomes still represent a very small fraction of the total (less than 1%) in old-age. Whether they are important in the functioning-of the tissue likely depends upon (a) whether distribution is even, or if instead there is mosaicism among neurons, and (b) the complement of other forms of mutation which also prevent transcription of mt-DNA. This is a joint project with Dr. C.F. Filburn of LBC, NIA.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00801-02 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Contractile Deficit of Rat Heart Cells to Norepinephrine With Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R-P. Xiao Clinical Associate LCS, NIA  
Others: E.G. Lakatta Chief LCS, NIA  
H.A. Spurgeon Research Physiologist LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In both humans and animals the effectiveness of  $\beta$ -adrenergic stimulation to augment the heart contraction strength declines with aging. We have investigated cellular mechanisms that may underlie the aging deficit. Twitch amplitude (TA), (%rest cell length), measured via photodiode array and cytosolic  $Ca^{2+}$  transient, indexed as transient in Indo-1 fluorescence ratio 410/940  $\mu$ m (IF) were measured in single ventricular myocytes of rats of varying age during electrical stimulation at 23°C. The L type  $Ca^{2+}$  current ( $I_{Ca}$ , pA/pF) was measured in additional cells during voltage clamp from -40 to 0 mV for 200 msec at 0.5 Hz (CsCl, 120, ATP, 3, and EGTA 10 mM in pipette; CsCl 5, and Ca 1 mM in bath). With aging the maximum TA, IF and  $I_{Ca}$  response to NE ( $10^{-7}$ M) are blunted.

	A (n=85, each age)		IF (n=85, each age)		$I_{Ca}$ (n=20, each age)	
	Control (C)	% C NE**	Control (C)	% C NE**	Control (C)	% C NE**
3 Mo	6.27 $\pm$ 0.32	222.73 $\pm$ 9.33	0.200 $\pm$ 0.009	193.77 $\pm$ 10.44	5.32 $\pm$ 0.53	235.83 $\pm$ 20.19
8 Mo	6.71 $\pm$ 0.30	174.38 $\pm$ 7.66	0.225 $\pm$ 0.012	170.17 $\pm$ 14.10	5.36 $\pm$ 0.42	179.46 $\pm$ 12.79
24 Mo	6.63 $\pm$ 0.44	139.00 $\pm$ 9.98	0.260 $\pm$ 0.012	144.36 $\pm$ 10.38	5.33 $\pm$ 0.61	156.71 $\pm$ 14.07
Age effect *p<0.02; **p<0.001.						

Thus, the blunted Ta and CaT responses to NE with aging are due to a failure to sufficiently augment  $Ca^{2+}$  influx via  $I_{Ca}$  to trigger  $Ca^{2+}$  release from the sarcoplasmic reticulum (SR) and to  $Ca^{2+}$  load the SR.

Combined into Z01 AG 00279-03 LCS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00802-02 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Physical Conditioning on Cardiovascular Aging Changes in Normal Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Fleg	Unit Chief	LCS, NIA
Others:	F.C. O'Connor	Statistician (Health)	LCS, NIA
	E.G. Lakatta	Chief	LCS, NIA
	A. Nussbacher	Visiting Fellow	LCS, NIA
	P. Vaitkevicius	Guest Researcher	LCS, NIA

COOPERATING UNITS (if any)

Division of Cardiology, Johns Hopkins Hospital (G. Gerstenblith, S. Schulman, L.Becker); Division of Geriatrics, University of Maryland (A. Goldberg, L. Lakatta).

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOXES

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

**A.** It is not known whether central or peripheral adaptations are primarily responsible for the markedly increased aerobic performance of endurance trained older men relative to their sedentary age peers. To answer this question, we performed maximal upright cycle ergometry in 16 endurance trained men 63±7 yr old and 35 untrained men of similar age from the BLSA. During cycle ergometry, trained men achieved higher maximal workloads, WL, (177±28 vs 131±28 watts, p<.0001) and higher peak VO<sub>2</sub> (34.2±3.6 vs 22.3±5.8ml/kg/min, 0<.0001). The higher peak VO<sub>2</sub> in trained men was achieved by a 22.5% higher cardiac index, CI, (9.8±1.9 vs 8.0±1.5l/min/m<sup>2</sup>, p<.001) and a 16.5% greater arteriovenous O<sub>2</sub> difference (13.3±2.6 vs 11.5±3.0 vol/100ml, p<.05). The higher maximal CI in the athletes was mediated entirely by a higher stroke volume index, SVI, (68.7±10.5 vs 57.5±11.3ml/kg/m<sup>2</sup>, p<.002). Thus, the augmented aerobic capacity of endurance trained older men during upright cycle exercise (EX) is achieved by both central and peripheral adaptations, which are of similar magnitude. **B.** It has been hypothesized that age-associated reductions in physical conditioning status mediates the decrease in LV early diastolic performance seen with advancing age. To test this hypothesis, we measured radionuclide ventriculographic peak filling (PF) rates at rest and throughout graded maximal upright cycle ergometry in 56 sedentary BLSA men and 12 highly trained old men. At rest, at 50% of maximum WL and at maximal effort, PF rates declined with age but were similar in older athletes and their sedentary age peers. These results suggest that the age-associated decline in early diastolic filling (DF) observed at rest and during EX cannot be reversed even by intensive long-term endurance training. **C.** To determine the effect of deconditioning on LV DF in endurance trained older men, 9 such men underwent serial radionuclide ventriculography at rest and during maximal upright cycle EX before and after 12 weeks of deconditioning. Although resting PF rates and cardiac volumes did not change significantly after deconditioning, at peak EX WL, PF rate (893±197 vs 1124±319ml/sec, p<.05) as well as end-diastolic volume index (73±13 vs 84±17, p<.05) and SVI (59±11 vs 71±14ml/m<sup>2</sup>, p<.05) were reduced after deconditioning. These findings suggest that the decrease in LV volumes at peak EX seen after deconditioning in highly trained seniors may be mediated by a reduction in diastolic early PF rate.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00803-02 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age-Associated Patterns of Gene Expression in the Heart

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Boluyt	NRC Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	L. O'Neill	Biologist	LCS, NIA
	A. Meredith	Stay-in-Schooler	LCS, NIA

COOPERATING UNITS (if any)

Department de Biologie Appliquee, Universite d'Auvergne Clermont, Aubiere, France  
(A. Younes)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Atrial natriuretic factor (ANF) is synthesized and secreted by atrial tissue in response to experimental or genetic hypertension. Recent studies have shown that ANF is produced and secreted by left ventricular tissue as well, and that ANF has direct effects on ventricular cardiac myocyte contraction. Since heart contractile function is altered during aging, and the senescent heart exhibits many other phenotypic and genotypic characteristics of the hypertensive heart, we tested the hypothesis that the atrial natriuretic factor (ANF) gene would be upregulated with advancing age. Since progressive age-associated myocyte hypertrophy is evident in left ventricle (LV) of the Wistar, but not in the LV of F344 nor in the right ventricle (RV) of either strain, total RNA was isolated from the LV and RV of male Wistar and Fischer rats aged 1.5-27 mo of age. Northern blots were probed with a radiolabeled cDNA probe synthesized by PCR using oligonucleotides complementary to the published sequence. The levels of mRNA coding for ANF increased progressively with advancing age in ventricles of both strains of rats. ANF mRNA abundance was 7-fold greater in ventricles of senescent compared to young adult rats. In freshly isolated ventricular myocytes, a similar pattern was observed. ANF mRNA levels were not augmented during aging in the atria of Wistar rats. In contrast to the age-associated increase in ventricular ANF mRNA levels, the concentration of ANF peptide in the LV decreased with advancing age, suggesting that either secretion or degradation rates are increased in the ventricles during aging. To obtain an indication of hemodynamic stress, expression of early response genes was assessed. Levels of both heat shock protein (hsp70) and c-jun mRNAs were elevated  $\approx$ 2-fold in hearts of some, but not all senescent rats, compared to those of young adult rats. c-fos and junB mRNAs were not elevated in any of the hearts studied. The low level induction of hsp70 and c-jun gene expression in some senescent hearts, suggests that a subpopulation of myocytes and/or fibroblasts may be stressed in the aging heart. Since in F344 LV and in the RV of both strains the age-associated elevation in ANF occurs in absence of myocyte hypertrophy, these results suggest that an independent age-related mechanism exists to regulate ANF gene expression in the ventricles. Further study is required to identify factors regulating ANF gene expression in the ventricles during aging, and to determine the fate of ANF peptide in the senescent heart.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00804-02 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cardiac Gene Expression During the Transition from Hypertrophy to Heart Failure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Boluyt NRC Fellow LCS, NIA  
Others: E.G. Lakatta Chief LCS, NIA  
M. Crow Senior Staff Fellow LCS, NIA  
L. O'Neill Biologist LCS, NIA  
A.L. Meredith Stay-in-Schooler LCS, NIA

COOPERATING UNITS (if any)

Boston VAMC, Boston, MA (O.H.L. Bing)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.1

OTHER:

0.4

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Heart failure is a progressive disease with extremely poor prognosis. The failing heart is characterized by impaired cardiac muscle function and increased interstitial fibrosis. The mechanisms initiating the transition from stable hypertrophy to irreversible heart failure are unknown. Our purpose was to determine whether the functional impairment of the failing heart is associated with changes in levels of mRNA encoding proteins responsible contraction and relaxation, and whether the increased fibrosis in the failing heart is related to induction of genes encoding extracellular matrix components. We studied hearts of 18-24 mo spontaneously hypertensive rats with signs heart failure (SHR-F) or without evidence of failure (SHR-NF), and from age-matched normotensive Wistar-Kyoto (WKY) rats. Compared to WKY, SHR-NF rats exhibited LV (2.2-fold), and RV (1.5-fold) hypertrophy, while SHR-F rats were characterized by comparable LV hypertrophy (2.1-fold) and augmented RV hypertrophy (2.4-fold; all  $p < 0.01$ ). In SHR-F hearts the level of  $\alpha$ -myosin heavy chain (MHC) mRNA was decreased in both ventricles to 1/3 and 1/5 of the SHR-NF and WKY values, respectively (both  $p < 0.01$ ). Levels of  $\beta$ -MHC, actin, and myosin light chains did not differ among the 3 groups in the LV. Levels of atrial natriuretic factor (ANF) mRNA were elevated 3-fold in the LV of SHR-NF rats ( $p < 0.05$ ), but were not increased in the RV of SHR-NF compared to WKY rats. During the transition to failure, ANF mRNA levels increased an additional 1.6-fold in the LV, and were increased 4.7-fold in the RV (both  $p < 0.05$ ). The levels of fibronectin (FN), pro- $\alpha 1(I)$  and pro- $\alpha 1(III)$  collagen (CN) mRNAs were not elevated in either ventricle of the SHR-NF group, but were 4-5-fold higher in both ventricles of SHR-F rats (all  $p < 0.05$ ). Transforming growth factor- $\beta_1$  (TGF- $\beta_1$ ) mRNA abundance was not elevated in ventricles of SHR-NF rats but increased 1.3-fold in the LV and 2-fold in the RV during the transition to heart failure (both  $p < 0.05$ ). The decrease in  $\alpha$ -MHC mRNA levels represents a pretranslational basis for the slowed contraction observed in cardiac muscle from failing hearts. The increase in FN and CN mRNA levels suggests that the observed increase in myocardial fibrosis in failing hearts is regulated at the level of gene expression. The increase in abundance of TGF- $\beta_1$  mRNA in conjunction with the upregulation of FN and CN genes suggests that activation of TGF- $\beta_1$  gene expression may be a mechanism initiating interstitial fibrosis during the transition from stable, compensated hypertrophy to overt heart failure.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00805-02 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Abnormal Myocyte  $Ca^{2+}$  Handling in A Rodent Model of Chronic Heart Failure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Mayoux	Visiting Fellow	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	H.A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Compensated myocardial hypertrophy occurring in response to chronic pressure overload usually progress to a decompensated state, with myocardial depression and cardiac pump dysfunction. The mechanisms involved in the transition from the chronically adapted to the chronically failing heart remain unclear. A major reason has been a relative lack of animal models of stable hypertrophy. We have developed a practical and reproducible model of cardiac hypertrophy that progresses to chronic heart failure, within three months, by banding the ascending aorta of young rats. Gradual left ventricular overload occurs as the animals mature, leading to a compensated left ventricular hypertrophy and subsequent cardiac failure. Animals with cardiac failure, identified by clinical symptoms (increased respiratory rate), presented pathophysiological alterations consistent with heart failure: high lung weight, hypertrophy of the left atria, right ventricle, and right atria. In addition most of the failure animals were observed to have pleural effusions. The first physiological study on animals provided by our experimental model show that the contractile behavior of myocytes isolated from failing hearts tended to be different under stressful conditions. Myocytes from failing hearts had reduced rates of twitch shortening and rates of relengthening, especially at high drive rates (2.5 Hz) or at elevated external calcium concentrations (6 mM), compared to control. Application of the different technology available in the laboratory, such as measures of intracellular calcium handling with Indo-1, molecular biology ..., to this model of progressive heart failure should be able to elucidate mechanisms that underlie the transition from compensated hypertrophy to chronic heart failure.

Combined into Z01 AG 00226-11 LCS.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00806-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Induction of Immediate-early Genes by Extracellular ATP in Cardiac Cells.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J-S. Zheng Staff Fellow LCS, NIA

Others: M.O. Boluyt NRC Fellow LCS, NIA  
E.G. Lakatta Chief LCS, NIA  
M.C. Crow Senior Staff Fellow LCS, NIA  
L. O'Neill Biologist LCS, NIA  
B. Ziman Biologist LCS, NIA

COOPERATING UNITS (if any)

Cardiac Function Section, LCS

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.6

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Norepinephrine (NE) activates processes of hypertrophy and proliferation in cardiac myocytes and fibroblasts, respectively, in conjunction with expression of immediate early genes (IEG). Since adenosine 5'-triphosphate (ATP) is co-released with NE from sympathetic nerve endings in the heart, we studied the effects of extracellular ATP on IEG expression in cardiac cells and investigated the intracellular mechanisms of IEG induction by ATP. In response to micromolar quantities of extracellular ATP, levels of IEG mRNA increased at 15 min, peaked 30 min (5-8 fold), and declined to baseline by 1 hr in both cell types. ATP increased intracellular  $Ca^{2+}$  concentration ( $Ca_i$ ) in cardiac myocytes (MYO) and fibroblasts (CAFB) loaded with Indo-1-am, whereas NE did not. The the potency order of ATP analogues for increasing  $Ca_i$  and c-fos mRNA levels was: ATP $\gamma$ S > ATP > ADP $\beta$ S = ADP > 2-met-ATP > AMP-PNP. Adenosine had no effect on either c-fos or  $Ca_i$  levels. In MYO, the ATP-induced increase in  $Ca_i$  and c-fos were inhibited by pretreatment with intracellular  $Ca^{2+}$  chelator, BAPTA-AM. However, pretreatment with BAPTA-AM did not inhibit the NE-induced increase in IEG in MYO. NE increased the rate of protein synthesis (incorporation of  $^{14}C$ -phenylalanine into TCA-precipitable protein) 2-3-fold, whereas ATP did not. In CAFB, overnight pretreatment with TPA or pretreatment with staurosporine for 30 min decreased the ATP-induced level of c-fos. Western blot analysis showed that ATP induced tyrosine phosphorylation of a 40-45-kDa protein. These data suggest that the ATP-induced increase in IEG occurs via activation of  $P_2$ -purinergic receptors in both cell types, and that in CAFB multiple second messenger systems are involved. In MYO, IEG-induction by ATP, but not NE, is calcium dependent, suggesting that ATP and NE activate IEG expression by different intracellular signalling mechanisms. Furthermore, these results suggest that induction of c-fos is not sufficient to activate hypertrophy in MYO.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00807-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Treatment of Restenosis After Angioplasty With Microtubule Stabilizing Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Kinsella Research Physiologist LCS, NIA  
S. Sollott Senior Staff Fellow LCS, NIA  
Others: L. Cheng, R. Pauly, R. Monticone, M. Kuzuya, M. Crow, M. Jenkins, J. Froehlich, E. Lakatta

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Significant improvements in the primary success rate of various medical and surgical treatments of atherosclerotic disease have been made in the last few years. Yet recurring failures continue in 30 to 50% of the patients after balloon angioplasty, bypass surgery, and endarectomy because of late restenosis of the treated vessel. The restenosis is a result of a complex series of fibroproliferative responses to the vascular injury that results in vascular smooth muscle cell (VSMC) proliferation, migration, neointimal accumulation, and secretion of extracellular proteins. Microtubules are likely involved in controlling or moderating critical intracellular mechanisms necessary for the VSMC fibroproliferative response. We found that taxol, an anti-tumor drug which stabilizes microtubules, inhibited VSMC proliferation, migration, and invasion in vitro. In vivo, taxol prevented neointimal VSMC accumulation in the rat carotid artery after balloon dilation and endothelial denudation injury. These experiments suggest that taxol or other pharmacologic agents that stabilize microtubules may have therapeutic value in preventing human restenosis after balloon angioplasty, bypass surgery, and endarectomy.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00808-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Age in Vascular Smooth Muscle Cell Migration and Invasion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Pauly	Senior Staff Fellow	LCS, NIA
Others:	E.G. Lakatta	Chief	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	R. Monticone	Biologist	LCS, NIA
	L. Cheng	Research Chemist, MBS	LCS, NIA
	Y. Gluzband	Chemist	LCS, NIA
	J. Fredman	IRTA	LCS, NIA
	G. M. Jenkins	Clinical Associate (MSF)	LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The development and progression of many vascular diseases depend on the migration and proliferation of vascular smooth muscle cells (VSMC) and their interaction with extracellular matrix (ECM). The incidence and prevalence of vascular disease increase with age, affecting approximately 50% of men (by age 65) and women (by age 75). We have found previously that proliferating VSMC aggressively degrade and invade a reconstituted basement membrane barrier (modeled to mimic the basement membrane which surrounds individual VSMC and separates them from endothelial cells in the form of the internal elastic lamina *in vivo*) in response to PDGF, while the migration and invasion of serum-starved/differentiated VSMC was less than 20% ( $p < 0.001$ ) that of proliferating cells. We demonstrated that VSMC migration through this ECM barrier requires 72 kD Type IV gelatinase. In this project we investigated the migratory/invasive, proliferative, and differentiative behavior of VSMC derived from young (age 3-6 mo) and old (age 24 mo) rats. Two populations of VSMC (neointimal and medial) were obtained following balloon catheter injury for each age group. Early passage ( $P_2$ - $P_3$ ) young neointimal VSMC exhibit 75% more migratory and invasive behavior as compared with ( $P_2$ - $P_3$ ) young medial VSMC. At later passages ( $P_6$ - $P_{10}$ ) young medial and young neointimal VSMC exhibit similar migratory and invasive characteristics. In contrast, old medial ( $P_2$ - $P_3$ ) show as aggressive migratory and invasive behavior as old neointimal ( $P_2$ - $P_3$ ). When VSMC from all four groups were growth arrested, their migratory and invasive behavior was less than 20% that of age/phenotype matched proliferating cells. We have observed differences in active 72 kD Type IV gelatinase and in receptor tyrosine kinase (RTK) activation in response to PDGF between proliferating and differentiated VSMC. Future studies employing gene markers of differentiated and proliferating VSMC such as calponin, CHIP 28 and Osteopontin as well as 72 kD Type IV gelatinase expression and activation should provide important information in understanding these age-associated behavioral differences in migratory/invasive behavior of VSMC.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00809-01 LCS

PERIOD COVERED

November 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dietary Fatty Acid Modulation of Myocardial Function and Influences on Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Pepe	Visiting Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	H. A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Laboratory of Cardiovascular Science

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The period of cardiac myocyte contraction, the transmembrane action potential and cytosolic  $Ca^{++}$  transient are extended in old rat cardiac cells compared to those from young adults. The rate of sarcoplasmic reticulum uptake of  $Ca^{++}$  and the level of  $Ca^{++}$  stimulate ATPase activity decreases with senescence and the cardiac response to  $\beta$ -adrenergic stimulation is reduced with aging. It is proposed that "normal" age-related changes in cardiac function and increased cardiac pathology with age may be associated with alterations to membrane composition which may be intervened by dietary lipid modification of myocardial cell and intracellular membranes. The vulnerability to arrhythmic stimuli increases with age. Short term feeding with n-3 polyunsaturated fatty acid (n-3 PUFA) rich diet in old rats and monkeys significantly reduces the incidence of ventricular arrhythmias whereas saturated fat rich diet (SAT) is pro-arrhythmogenic. SAT diet induces a marked increase in myocardial  $O_2$  demand independent of contractile function under control conditions. In contrast  $O_2$  demand was very low following n-3 PUFA diet. Coronary vasodilator reserve was greater following n-3 PUFA diet than SAT diet. These differences are not due to any change in basal metabolism or vascular function but rather to intracellular  $Ca^{++}$  homeostasis. The present study (commenced January 1993), investigates the effect of dietary lipid modulation in isolated cardiac myocyte membranes in order to test whether total intracellular  $Ca^{++}$  or  $Ca^{++}$  redistribution between the cytosol and organelles is altered. Cytosolic free calcium fluorescence (indo-1) and twitch amplitude of electrically stimulated isolated cardiac myocytes are currently being measured from n-3 PUFA or saturated fat dietary treated rats (6,12,24 mo). The responsiveness to the stresses of high and low stimulation rates (0.5, 2Hz), BAYK8644 (L-type  $Ca^{++}$  channel agonist), isoproterenol ( $\beta$ -adrenergic receptor agonist) and hypoxia is also to be assessed in these groups. Cell membrane fatty acid profile analysis is currently in progress to confirm the extent of change to cardiac membrane lipid composition.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00811-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy of Coronary Artery Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. C. Capogrossi	Medical Officer	LCS, NIA
Others:	J. Mühlhauser	Special Volunteer	LCS, NIA
	L. Cheng	Research Chemist	LCS, NIA
	M. Jenkins	Clinical Associate (MSF)	LCS, NIA
	A. Passaniti	Senior Staff Fellow (RMS)	LBC, NIA
	R. Pili	Visiting Fellow	LBC, NIA
	R. G. Crystal	Special Volunteer	PB, NHLBI
	P. Lemarchand	Visiting Fellow	PB, NHLBI

COOPERATING UNITS (if any)

Surgical Neurology Branch, NINDS (J. Merrill); Laboratory of Animal Surgery, NHLBI (M. Jones); Laboratory of Histology (T. Faraggiana) and Department of Vascular Surgery (S. Camilli), IDI, Rome, Italy

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

4.0

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene therapy may represent a novel approach for the treatment of myocardial ischemia. This research project aims at developing adenoviral vectors to transfer the cDNA for endothelial cell growth factors into cardiac cells. The same adenoviral vectors will be used for two different studies: (1) Angiogenesis and improvement of coronary collateral circulation: Neovascularization is expected to improve blood flow to ischemic areas of the myocardium. For this study the adenoviral vectors will be injected into the coronary circulation or directly into the myocardium. (2) Restenosis after angioplasty: Rapid reendothelialization of a segment of coronary artery which has undergone endothelial denudation during angioplasty may be expected to decrease the severity of restenosis and intimal hyperplasia. For this study the adenoviral vectors will be delivered to the localized area of the coronary artery which has undergone balloon dilatation. We have constructed adenoviral vectors which carry the cDNA for the following angiogenic growth factors. (1) Vascular endothelial growth factor (VEGF) (2) Acidic fibroblast growth factor (aFGF). (3) A recombinant form of aFGF which has been modified with the addition of the secretory signal sequence from PGF 4 (sp-aFGF). Unlike the natural form of aFGF this recombinant form of aFGF is secreted into the extracellular space.

Our initial studies show all three adenoviral vectors produce a functional protein capable of inducing endothelial cell growth and differentiation in vitro. In additional studies with an adenoviral vector which carries the cDNA for the reporter gene lacZ (AdRSV.lacZ) we have examined whether adenoviral vectors can transduce cardiac cells in the minipigs. We have found that intracoronary injection of Ad RSV.lacZ transduces endothelial cells, vascular smooth muscle cells and myocardial cells. In contrast, intramyocardial injection of AdRSV.lacZ transduces mostly myocardial cells. Studies are now in progress to further characterize the properties of the vectors which carry the cDNA for the angiogenic factors prior to their use in in vivo models of myocardial ischemia and restenosis after angioplasty.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00812-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Free Radicals on Endothelial Cell  $Ca^{2+}$  Homeostasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Corda	Visiting Fellow	LCS, NIA
Others:	M. C. Capogrossi	Medical Officer	LCS, NIA
	R. C. Ziegelstein	Guest Researcher	LCS, NIA
	H. A. Spurgeon	Physiologist	LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.8

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Free radicals are major determinants of oxidant injury during inflammation and post-ischemic reperfusion. Vascular endothelium is both a source and a target of reactive oxygen species. One such reactive oxygen species, hydrogen peroxide ( $H_2O_2$ ), may cause endothelial cell damage by affecting intracellular signal transduction mechanisms and disrupting  $Ca^{2+}$  homeostasis. The aim of the project is: 1) to characterize the effects of exposure of human aortic endothelial cells (HAEC) and human umbilical vein endothelial cells (HUVEC) to  $H_2O_2$  in the range 0.1mM-1mM on endothelial cell  $Ca^{2+}$  homeostasis; 2) to define the role of cytosolic  $Ca^{2+}$  concentration,  $[Ca^{2+}]_i$ , in the pathophysiology of oxidative injury. In order to detect the effect of hydrogen peroxide on HAEC and HUVEC  $[Ca^{2+}]_i$ , indo-1 AM loaded endothelial cells grown on glass coverslips were bathed in a Hepes buffer (CaCl<sub>2</sub> 1.5mM, pH 7.4) containing  $H_2O_2$  in the range 0.1mM-1mM.  $H_2O_2$  slowly increased  $Ca^{2+}$  and this effect was not reversible with wash-out. The increase in  $[Ca^{2+}]_i$  persisted in a  $Ca^{2+}$ -free solution with 1mM EGTA suggesting that extracellular  $Ca^{2+}$  does not contribute to the increase in  $[Ca^{2+}]_i$ . In other experiments the effect of  $H_2O_2$  on endothelial cell  $[Ca^{2+}]_i$  was examined following exposure to 1μM bradykinin, an agonist known to release  $Ca^{2+}$  from intracellular stores. Under these conditions,  $H_2O_2$  still produced an increase in  $[Ca^{2+}]_i$ . Pre-exposure of HAEC to thapsigargin, an inhibitor of microsomal or endoplasmic reticulum (ER)  $Ca^{2+}$ -ATPase which releases  $[Ca^{2+}]$  from the endoplasmic reticulum, abolished the response to bradykinin (1μM) but did not suppress the subsequent response to  $H_2O_2$ . These results suggest that  $H_2O_2$  releases  $[Ca^{2+}]$  from a thapsigargin-insensitive intracellular store and increases  $[Ca^{2+}]_i$  via this mechanism.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00813-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Molecular Characterization and Therapeutic Interventions for Restenosis**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Cheng Research Chemist LCS, NIA  
M. Jenkins Clinical Associate LCS, NIA

Others: S. Sollott, J. Kinsella, J. Froehlich, C. Bilato, M. Crow, M. Capogrossi, C. Nater, P. Heller, E. Lakatta

COOPERATING UNITS (if any)

Department of Biomedical Engineering, Johns Hopkins School of Medicine (Dr. Kam Leong)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Percutaneous Transluminal Coronary Angioplasty (PTCA) has become a widely used procedure for the treatment of coronary artery disease (CAD). However, restenosis at the site of PTCA remains a persistent problem. Restenosis following PTCA involves a fibroproliferative response to vascular injury and numerous attempts to modify this response, either through pharmacological interventions or mechanical devices, have met with very limited success. It is felt that by understanding the cellular and molecular mechanisms that underlie this fibroproliferative response to injury, more effective strategies to prevent restenosis can be explored. One mechanism that has been shown to be important in restenosis in the rat is the migration of vascular smooth muscle cells (VSMCs) from the media of the vascular wall to the intima. Recent studies by LCS scientists in cultured VSMCs suggest that the MMP-2 Type IV metalloproteinase (MMP-2) may be important in this process. Using gelatin zymography on extracts from carotid artery tissue isolated at varying time points following injury, we previously demonstrated the presence of the MMP-2 protein. To further clarify the in vivo relevance of this enzyme to the arterial response to injury, experiments involving the rat carotid injury model were performed. De-endothelialization was performed with an embolectomy catheter and vessels were subsequently harvested at the appropriate time points. Preliminary findings with immunocytochemistry, in situ hybridization studies, and the ribonuclease protection assay reveal parallel results. The mRNA and protein for the MMP-2 gene was expressed at moderate levels in uninjured vessels and decreased in expression a early time points. Within 5-7 days following injury expression increased and peaked at 14-21 days. The functional significance of these changes in vivo is presently under investigation. Treatment for restenosis is dependent on the development of an effective means of intravascular site-specific delivery. We recently began studies using a unique sustained-release biodegradable microcapsule. Initial experiments using Texas-red labeled albumin (TRA) incorporated into the microcapsules showed penetration of the TRA into the media in vessels from which the adventitia had been removed. Based upon preliminary results obtained about the potential role of MMP-2 in VSMC migration, we initiated in vivo studies using a peptide inhibitor of metalloproteinase activity. The peptide inhibitor was incorporated into the microcapsule and applied to the outside of the rat carotid artery that had undergone vascular injury. The results of these experiments are pending.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00814-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormonal Requirements for Intimal Thickening Following Vascular Injury in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Jenkins	Clinical Associate	LCS, NIA
	L. Cheng	Research Chemist	LCS, NIA
Others:	J. P. Froehlich	Chief, MB	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	C. Nater	Biological Science Lab Tech	LCS, NIA
	E. G. Lakatta	Chief	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The fibroproliferative response of vascular smooth muscle cells (VSMCs) to injury is regulated by the combined action of both autocrine and paracrine growth factors. In addition, hormonal factors circulating in the blood plasma are important for cellular growth of VSMCs in tissue culture. In vivo, a similar paradigm seems to exist after vascular injury where growth factors that are locally produced act in conjunction with plasma factors to induce proliferation. It has been shown that hormonal factors dependent on the pituitary gland are involved in VSMC proliferation and migration after arterial injury. Injury-induced neointimal formation is inhibited in hypophysectomized rats. Indeed, atherosclerosis or restenosis may in part be mediated by a complex endocrine modulation.

Hypophysectomy is a severe intervention and causes alterations in a number of hormonal factors; therefore, the exact mechanism(s) or factor(s) dependent on the pituitary gland is not known. In an attempt to further clarify this issue and pinpoint specific hormonal factors necessary for intimal thickening, we made rats specifically hypothyroid by dietary manipulations. Preliminary experiments show that rats fed thyroid suppressive diets (thiouracil-containing) exhibit a markedly diminished response to neointimal formation following balloon angioplasty. This blunted response was observed at 8, 14 and 21 days following vascular injury with the most significant inhibition occurring at 21 days. This also occurred in animals which, in addition to being rendered hypothyroid were made hypercholesterolemic. These observations suggest that the anti-proliferative effect of hypophysectomy on neointima formation is in part specifically mediated by a deficiency in thyroid hormone.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00815-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Signal Transduction Pathways Involved in Vascular Smooth Muscle Cell Migration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Crow	Senior Staff Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	R. Pauly	Senior Staff Fellow	LCS, NIA
	C. Bilato	Guest Researcher	LCS, NIA
	R. Monticone	Biologist	LCS, NIA
	Y. Gluzband	Chemist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular diseases. We have previously shown that in vitro VSMC migration in response to PDGF is suppressed in differentiated VSMCs and seek to identify differences in intracellular signalling between differentiated and proliferating VSMCs that may account for this suppression. Differentiated VSMCs retain their ability to respond to PDGF and upregulate expression of the immediate early response genes, c-fos and MCP-1 (JE) when stimulated by PDGF. Unlike proliferating cells, however, PDGF-stimulated differentiated VSMCs fail to activate calcium/calmodulin-dependent protein kinase (CamKinase) II activity. Blocking CamKinase II activation blocked the migration of proliferating VSMCs by more than 90%. In contrast, inhibitors of protein kinase C have no significant effect on migration. Pretreatment of differentiated cells with ionomycin (1  $\mu$ M) or endothelin (10-100 nM) (both of which are expected to increase intracellular calcium) resulted in an 84 + 6% return to the migration rate of proliferating VSMCs. This return was also blocked by CamKinase inhibitors and was unaffected by inhibitors of PKC. These results suggest that activation of CamKinase plays an important role in VSMC migration and the failure to activate it in differentiated VSMCs may be responsible for the suppression of migration. The most direct intracellular pathway by which PDGF could activate CamKinase II is through the activation of phospholipase C $\gamma$  (via tyrosine phosphorylation) following its association with the activated PDGF receptor. Immunoprecipitation of PLC $\gamma$  followed by blotting of the protein with an antibody to phosphotyrosine residues indicates that, in contrast to proliferating VSMCs, differentiated VSMCs do, in fact, fail to activate PLC $\gamma$ . Recent experiments have identified a role for basic FGF in the migration of VSMCs and suggest that PLC $\gamma$  activated by PDGF may be indirect and mediated through the FGF receptor. Our results focussing on PDGF intracellular signalling have identified at least one critical difference in the way in which proliferating and differentiated VSMCs respond to PDGF and have demonstrated that to activate differentiated VSMCs requires concurrent action by at least two growth factors/cytokines. This requirement may limit the response of VSMCs to injury to selected group capable of responding to agents.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00816-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nuclear Transcription and Skeletal and Cardiac Muscle Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Crow Senior Staff Fellow LCS, NIA

Others: E. Lakatta Chief LCS, NIA  
M. Boluyt NRC Fellow LCS, NIA  
N. Papadopoulos Visiting Fellow (DOD 3/1/93) LCS, NIA  
X. Long Visiting Fellow LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A significant component of musculoskeletal frailty in aging animals is diminished contractile capacity. The relatively high level of protein accumulation required for the assembly of the contractile apparatus requires specialized systems to selectively stabilize the proteins and mRNA encoding them and a high level of sustained transcriptional activity driven by muscle-specific and ubiquitous nuclear transcription factors. These studies examine three such factors that are present in relatively high levels in both cardiac and skeletal muscle and that are known to drive expression of a number of muscle-specific genes. These are 1) the serum response factor (SRF), 2) a zinc-dependent DNA-binding protein that recognizes G-rich sequences, and 3) the thyroid hormone receptors. The first two factors are involved in the transcription of a number of muscle-specific genes, including sarcomeric actins, the myosin light chains, myoglobin, and the muscle-specific isoforms of creatine kinase. Reagents are being developed to measure their levels of expression in tissues from young and aging animals. The thyroid receptors also act as nuclear factors involved in tissue-specific expression of specific contractile genes. Their importance to aging lies in the fact that, in terms of gene expression, aging resembles a hypothyroid state. Activation of the genes, such as the b-myosin heavy chain, that are associated with hypothyroidism may have important functional consequences. Since numerous have failed to provide a consensus on whether thyroid hormone levels actually change with age, we have focussed on possible changes in the level of expression or the types of receptors expressed during. Our preliminary indication are that there are selective decreases in one thyroid receptor subtype with aging that could account for the changes seen in gene expression. A precise understanding of the molecular mechanisms by which cardiac and skeletal muscle-specific gene expression is initiated and sustained provides the background for understanding age-associated changes in gene transcription that may contribute to musculoskeletal frailty in the aging.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AG 00817-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Vascular Smooth Muscle Cell Function and Age-Related Hypertension**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. P. Froehlich	Chief, MBS	LCS, NIA
	E. Koh	Visiting Fellow (DOD 1/11/93)	LCS, NIA
	Y. Miyashita	Visiting Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	J. L. Kinsella	Research Physiologist	LCS, NIA
	S. J. Sollott	Senior Staff Fellow	LCS, NIA
	S. Pepe	Visiting Fellow	LCS, NIA
	A. Bagrov	Visiting Associate	LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Behavioral Sciences

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Peripheral vascular resistance becomes elevated with age increasing the risk of cardiovascular disease. The physiologic changes in the vasculature that accompany aging closely resemble those seen in younger individuals with essential hypertension. The etiology of this condition may involve a defect in intracellular  $Ca^{++}$  metabolism. We investigated this hypothesis in a rat model for hypertension using freshly-isolated arterial smooth muscle cells (SMC) which retain their *in vivo* phenotype. Mean systolic blood pressure showed a significant elevation between 6 and 30 months of age (131 mm ~~vs~~ 155 mm;  $p < .001$ ). Old SMC showed transiently higher resting intracellular  $Ca^{++}$  levels compared to young cells following exposure to different extracellular  $Ca^{++}$  loads. Blockade of T-type  $Ca^{++}$  channels by  $Ni^{++}$  was more effective in lowering resting  $Ca^{++}$  in young than in old SMC, whereas no age difference was found with respect to  $Ca^{++}$  sequestration by sarcoplasmic reticulum (SR). Stimulation of SMC with the  $\beta$ -agonist isoproterenol caused the redistribution of  $Ca^{++}$  from the SR to the extracellular compartment. Measurements of the rate of isoproterenol-dependent  $Ca^{++}$  removal from the SR showed that SMC from old rats retain significant levels of  $Ca^{++}$  over longer periods of time than young SMC. Increased SR  $Ca^{++}$  stores following  $\beta$ -agonist stimulation could result in diminished smooth muscle relaxation and increased vascular tonus contributing to the development of hypertension in older animals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00819-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of  $Ca^{2+}$ /Calmodulin-Dependent Protein Kinase II in Heart  $Ca^{2+}$  Channel Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R-P. Xiao Visiting Associate LCS, NIA

Others: E.G. Lakatta Chief LCS, NIA

COOPERATING UNITS (if any)

Department of Physiology, University of Maryland School of Medicine, Baltimore (H. Cheng, J. Lederer); Department of Biochemistry, Nagoya City University School of Medicine, Nagoya, Japan (T. Suzuki)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

$Ca^{2+}$  current flowing via sarcolemmal  $Ca^{2+}$  channels of heart cells is an important determinant of cell  $Ca^{2+}$  homeostasis and thus an important determinant of a variety of cell functions. While  $Ca^{2+}$ - and membrane voltage-dependent positive regulation of  $Ca^{2+}$  channels have been recently reported, little is known of the mechanism. Our studies provide compelling evidence that in adult rat cardiac ventricular cells  $Ca^{2+}$ /calmodulin dependent kinase II mediates L-type  $Ca^{2+}$  current facilitation, i.e., an augmentation in current magnitude and or slowing of its inactivation during repetitive depolarizations, by single strong depolarizing prepulse or by depolarizing holding potentials. All of these effects on the current were completely abolished by a inclusion of specific peptide inhibitor of  $Ca^{2+}$ /calmodulin kinase II within the pipette filling solution or by the replacement of  $Ca^{2+}$  with  $Ba^{2+}$  in bath solution. The involvement of  $Ca^{2+}$ /calmodulin kinase II on  $Ca^{2+}$  current regulation is further supported by the localized distribution of a specific antibody to the kinase near cell sarcolemma as revealed by digital confocal fluorescent imaging. Furthermore, the immunofluorescence of the antibody was significantly enhanced by depolarization with high  $[K^+]_o$  and attenuated by removal of  $Ca^{2+}$ , or by W7, a calmodulin inhibitor. Thus,  $Ca^{2+}$ /calmodulin kinase II dependent protein phosphorylation is an important mechanism by which variable factors, such as  $Ca^{2+}$ , repetitive stimulation and strong depolarization can positively regulate  $Ca^{2+}$  current in heart cells, and that membrane potential plays an essential role in the modulation of the kinase activity. These findings provide new insights toward understanding  $Ca^{2+}$  channel regulation in cardiac cells as possibly in other cell types as well.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00820-01 LCS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Novel Cardiac Myofilament Desensitizing Substance Released by Endocardial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Shah Guest Researcher

LCS, NIA

Others: A. Mebazza Guest Researcher

LCS, NIA

E. Lakatta Chief

LCS, NIA

COOPERATING UNITS (if any)

Pulmonary Anesthesiology Laboratory, Johns Hopkins Hospital (R.C. Wetzel),  
Department of Cardiology, Johns Hopkins University (J.L. Robotham)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vascular endothelium regulates smooth muscle tone through the coordinated release of agents such as prostacycline, endothelium-derived relaxing factor (EDRF) or nitric oxide, and endothelin. Recent studies, both in isolated cardiac multicellular preparations and in intact hearts in vivo, indicated that endocardial endothelium may similarly influence myocardial contraction predominantly by modulating the onset of relaxation. Bioassay studies using cultured endocardial endothelial cells and isolated cardiac papillary muscle preparations suggest that diffusible agents are involved, one of which is probably nitric oxide. However, the relative contribution of endocardial endothelial and coronary vascular endothelial cells to these effects, and the myocardial mechanism of action of substances released by these cell types is unclear. We studied the effects of effluent of superfused endocardial and vascular endothelial cultured cells on contraction and intracellular calcium transients of isolated adult rat cardiac myocytes. Both endocardial and vascular endothelial cells tonically released a novel substance which rapidly and reversibly decreased the amplitude of myocytes twitch contraction by inducing earlier relaxation, and also increased diastolic cell length. These effects were not associated with any change in the intracellular calcium transient, indicating cardiac myofilament "desensitization". The activity of endothelial cell effluent remained stable at 37°C for several hours or at 4°C for at least 48 hours. The action of this substance did not involve nitric oxide, cyclic GMP or prostanoids, nor changes in intracellular pH. These properties suggest that endothelial cells may rapidly modulate cardiac contraction-relaxation coupling and diastolic tonus by altering myofilament properties, as well as exert distant effects because of the unusual stability of this substance.





Name and Number:                   JOHNS HOPKINS UNIVERSITY (NO1-AG-01-2118)

Title: Vascular Stiffness, Arterial Pressure, and Cardiac Mass with Aging in a Genetically Homogeneous Population that Differs in Lifestyle

Date Contract Initiated: 1990

Current Annual Level: \$255.093

With advancing age, arterial stiffness increases and is accompanied by increased systolic pressure and mild left ventricular hypertrophy. This study addresses how arterial stiffness affects the myocardium. Does, for example, the increased systolic pressure cause the increase in heart size or is it a consequence? Are the changes normal, or are they absent in a population where arterial stiffness does not increase? A previous study of two Chinese populations published in 1985 which identified different degrees of change in vascular stiffness dependant on diet and lifestyle, but did not address cardiac changes. This study is investigating two populations in Taiwan. One population is in an isolated rural area and another in an urban setting where diet, exercise habit, and stress is much different. The field portion of this study is completed, and the data analysis phase begun. Approximately 2200 subjects ranging from 30 to over 70 years, equally divided by sex and by presence/absence of hypertension have been studied, with approximately half the subjects from the rural locale. Lifestyle and diet questionnaires, pulse wave velocities, and echocardiographic cardiac dimensions have been quantified. The epidemiologic analysis portion of the study is now proceeding, with a planned completion date of early 1994. Because of the genetic homogeneity of these two populations, the question of cause/effect relating cardiac mass and vascular stiffness can now be resolved.

### Objectives:

This study seeks to determine whether the age associated increase in vascular stiffness is the cause of or results from increased cardiac mass. Because diet, exercise habits, and lifestyle are potent modulators of blood pressure change with advancing age, an increased understanding of these important factors and their role in modulating age-associated cardiac hypertrophy and vascular stiffness will result.

### Methods Employed:

Subjects from an isolated rural community (Quemoy), and from a geographically isolated urban population (Puli), both on the island of Taiwan are selected based on age, sex, and freedom from cardiovascular disease except hypertension. In addition, adequacy of historical medical records is used as a selection criteria. A lifestyle and diet questionnaire and a, comprehensive physical exam are performed. Subjects are stratified into equal sized age and sex matched populations as either hypertensive or free of hypertension. The subjects thus retained are then studied using applanation tonometry and 2D echocardiography to determine pulse wave velocity and ventricular dimensions. From this information, plus blood pressure, vascular stiffness can be calculated using techniques validated during the initial phases of the study, by direct comparison with invasive cardiac catheterization done simultaneously.

### Major Findings:

The field phase of the study has just concluded. The population goals for age and sex matched individuals in both normotensive and hypertensive groups have been met

Significance to Biomedical Research and to the Program of the ICD: Despite great strides in understanding and treatment of heart and vascular disease, the precise



interactions between these two important areas are not fully understood. The results of this study should enable us to state definitively whether the cardiac changes seen in aging are a result or are the cause of the changes in the vasculature. This intern has broad implications in care and treatment. The analytic phase, recently begun, should be completed in the first quarter of 1994, and the study concluded. Until that data is completely analyzed, the next steps cannot be defined. The measurements obtained in this study will be compared with a comparable set of measurements taken from the BLSA

Publications: none



Name and Number: JOHNS HOPKINS UNIVERSITY (N01-AG-4-2109)

Title: Non-Invasive Assessment of Cardiac Structure and Function in Aging Men and Women

Date Contract Initiated: October 1, 1989

Current Annual Level: \$268,327

during the past 14-2 years, rest and exercise thallium and gated blood pool cardiac scans have been performed respectively on nearly 900 and 450 participants in the Baltimore Longitudinal Study on Aging (BLSA). These studies have provided unique insights concerning the prevalence and prognostic significance of exercise-induced myocardial blood flow abnormalities (i.e. ischemia) as well as the effect of age, gender, life-style and disease on cardiac structure and function at rest during aerobic exercise. The present contract is in the fifth year of a 9 year renewal, during which cardiac blood pool and thallium scans are being continued in the groups of individuals listed below.

Although the nuclear cardiac studies are performed at Johns Hopkins Hospital, conceptualization of the specific research questions, the selection of study subjects, and the data analyses are the responsibility of the LCS. Drs. Jerome Fleg and Edward Lakatta direct the overall research effort and review the collected data and plan data analyses with statisticians from our laboratory. The exercise studies are performed under the supervision of Dr. Gary Gerstenblith and the scans are read by Dr. Lewis Becker, both from the Johns Hopkins Cardiology Division. This long-standing collaboration continues to bear fruit as evidenced by the list of derived publications shown.

1. Longitudinal Study of Cardiac Function. In 100 BLSA men and women who have had blood pool scans at least 5 years previously, the test is repeated, with simultaneous measurement of oxygen consumption ( $\text{VO}_2$ ). This will allow insight into longitudinal changes in cardiac function at rest and during exercise. The  $\text{VO}_2$  data will provide information regarding central (cardiac) versus peripheral (arteriovenous oxygen difference) mechanisms for maintaining  $\text{VO}_2$  with advancing age. Forty repeat studies have been performed to date.

2. Cardiac Function in Highly Trained Seniors, Sedentary Subjects Pre- and Post-Training, and Obese Individuals Pre- and Post-Weight Loss. These scans, all performed with simultaneous  $\dot{V}O_2$  measurements, during maximal upright cycle exercise allow determination of the central and peripheral effects of conditioning status and obesity on aerobic exercise performance, and the interrelations of such lifestyle variables with the aging process. These studies are performed in collaboration with investigators from the Academic Teaching Nursing Home project of the University of Maryland. Analysis in 16 senior athletes suggests that central (cardiac) and peripheral factors (arteriovenous oxygen difference) account nearly equally for the age-related decline in peak  $\dot{V}O_{2\max}$ . Nine men have undergone serial scans before and after detraining. These men demonstrate reduction of maximal cardiac index, mediated by reduced end-diastolic and stroke volume indices as well as peak filling rate after 12 weeks of detraining.

3. Cardiac Fraction in Patients with Latent Coronary Artery Disease (CAD). Data from such individuals with concordant abnormal exercise ECG's and exercise thallium scans, suggest that ischemia and advanced age have additive effects on certain cardiac parameters. Extension of these studies allows more accurate characterization of this interaction between age and silent CAD and help to clarify discrepancies in the cardiovascular aging literature which have resulted from the inclusion of such individuals with silent CAD in some studies but not others. Our findings to date indicate that both age and silent (CAD) cause similar but additive blunting of the ejection fraction response and greater increases in end diastolic volume index (EDVI) during exercise.



4. Cardiac Function in Patients after Cardiac Transplantation. Stable outpatients who are at least six months past cardiac transplantation undergo rest and exercise scans with simultaneous measurement of  $\text{VO}_2$ . By comparing the responses of these patients with age- and gender-matched BLSA normals, we will gain insights regarding the effect of cardiac denervation on the cardiovascular response to exercise. Thus far, in 10 transplant recipients, higher heart rates and lower left ventricular volumes and ejection fraction have been observed at rest; although the heart rate and blood pressure responses to upright cycle exercise are reduced, the responses of cardiac volumes and ejection fraction to exercise resemble those of age-matched BLSA controls.

5. Additional Studies in Normals with simultaneous measurement of  $\text{VO}_2$ . In other BLSA volunteers who have no evidence of CAD by all available criteria, MUGA scans are made:

a. To contrast the effect of age in men with its effect in women on the cardiovascular response to stress. Our results in 200 healthy subjects suggest that age-associated cardiac dilatation to preserve stroke volume occurs in men but not women, whereas older women have higher heart rate responses than older men during upright cycle exercise.

b. To evaluate the effect of age on the response to other commonly employed drugs used to treat large numbers of elderly individuals, e.g., vasodilators. Such a study, using sodium nitroprusside to reduce cardiac afterload at rest and throughout maximal cycle exercise, has recently begun.

c. To provide a base of 350-400 individuals for a longitudinal evaluation of the effect of age itself on cardiovascular function.

#### B. Thallium Perfusion Scans

Our initial exercise thallium studies in apparently healthy BLSA volunteers demonstrated that the combination of an abnormal exercise ECG and a thallium perfusion defect occurred primarily in older men and predicted the development of coronary events in 48% of these subjects over a 4.6 years mean follow-up period. A preliminary analysis of coronary risk in highly trained men aged 55-79 indicates a prevalence of concordant abnormal exercise ECG and thallium scans identical to that in age-matched sedentary BLSA men. An analysis of the risk factors for silent myocardial ischemia in apparently healthy BLSA and NIH volunteers demonstrated that older age, male sex, reduced HDL-cholesterol and increased waist-hip ratio were independent predictors of silent ischemia.

Participants in the BLSA would continue to undergo exercise thallium tests as they become eligible to do so in the next 4 years. This would include those who become 40 years of age, those who enter the program, and those who are capable of undergoing a treadmill test but who for one reason or another did not have a thallium test during the past 5 years. This would enable us to continue to identify asymptomatic reversible ischemia in the BLSA participants and allow us to better assess the accuracy of a positive test in asymptomatic individuals in predicting the future development of clinical ischemic events. In addition, repeat thallium scans will be initiated on subjects who last underwent such scans at least 10 years previously. These repeat scans will allow assessment of the longitudinal development and progression of both latent and overt CAD, identification of risk factors associated with disease progression and determination of the prognostic significance of longitudinal changes in these perfusion scans. To date 41 such longitudinal thallium scans have been performed.

The following abstracts summarize the scientific program that has resulted from the contract during FY92:

Katzel LI, Lakatta L, Schulman SP, Drinkwater DT, Becker LC, Muller D, Fleg JL. Does regular vigorous exercise protect against occult coronary artery disease in older men? *Circulation* 1992; 86(4)Suppl. 1: I-672

Schulman SP, Goldberg AP, Becker LC, Lakatta L, Coombs V, Gerstenblith G. Cardiac response to deconditioning in older athletes. *J Am Coll Cardiol* 1992; 19: 386A





Sorkin JD, Katzel LI, Busby MJ, Lakatta L, Fleg JL. Metabolic risk factors for exercise induced silent myocardial ischemia in apparently healthy men. *Circulation* 1992, 86(4)Suppl. I: I-325

Publications:

Fleg, JL. Prevalence and prognosis of exercise-induced silent myocardial ischemia in apparently healthy older subjects. In: Orimo H, Fukuchi Y, Kuramoto K, Iriki M, eds. *New Horizons in Aging Science, The Organizing Committee of the Fourth Asia/Oceania Regional Congress of Gerontology, 1992*;152-53.

Fortney S, Tankersley C, Lightfoot JT, Drinkwater D, Clulow J, Gerstenblith G, O'Connor F, Becker L, Lakatta E, Fleg J. Cardiovascular responses to lower body negative pressure in trained and untrained older men. *J Appl Physiol* 1992; 73:2693-2700.

Fleg JL, Gerstenblith G, Lakatta EG. Pathophysiology of the aging heart and circulation. In: Messerli FH, ed. *Cardiovascular Disease in the Elderly*, 3rd Edition 1993;27-58.

Fleg JL, Schulman SP, Gerstenblith G, Becker LC, O'Connor FC, Lakatta EG. Additive effects of age and silent myocardial ischemia on the left ventricular response to upright cycle exercise. *J Appl Physiol*, 1993; in press.

Katzel LI, Ragoobarsingh N, Fleg JL, Paidi M, Goldberg AP. Apolipoprotein E4 polymorphism increases the risk for exercise-induced silent myocardial ischemia in older men. *Arteriosclerosis and Thromb*, 1993; in press



LN

LCMB

ICP

LMG

LPC



## Laboratory of Cellular and Molecular Biology

This laboratory conducts fundamental research on some of the basic systems of molecular biology as well as studies designed to understand the biology of aging. The laboratory was not designed to operate in a pyramidal mode, in which the laboratory chief formulates a grand design. Rather it was organized to bring together sections led by investigators with a diversity of goals but also a community of interests. Every section in the laboratory has engaged in successful collaborations with other sections.

The Inorganic Biochemistry Section has conducted many studies on nucleic acid structure and function as well as a variety of studies on molecular structural changes in aging. A particular emphasis at the moment is the project on the mechanism of *E. Coli* RNA synthesis at the site of internucleotide bond formation, leading to a model of structure in the active site of RNA polymerase that is compatible with the functions of the enzyme. A comprehensive theory has been developed for the high fidelity of transcription. Studies have been initiated with eukaryotic (yeast) RNA polymerase.

The Unit on In Vivo NMR focuses on the physiological studies related to aging, involving the BLSA and develops new techniques needed to improve the physiological studies. Solid-state NMR is used to study structure of a  $\beta$ -amyloid of importance in Alzheimer's disease.

The Molecular Dynamics Section shares the commitment to structural studies with the Inorganic Biochemistry Section, and is actively collaborating on the RNA synthesis study. Its importance is signified by its title - the emphasis on the dynamics, i.e., molecular motion that occurs during - and is required for - biological function. The section is presently involved in characterizing the dynamics of the interaction of hemoglobin with oxygen and other ligands, having identified distal perturbations and subunit interactions across the  $\alpha\beta$  interface as important components in the process. It is also engaged in studying the consequences of the discovery of enhanced hemoglobin oxidation rate under hypoxic stress resulting in the formation of superoxide.

The Macromolecular Chemistry Section has carried out a variety of activities designed to understand the molecular basis of drug action and to lead to the design of better drugs. Recent work has been focused on cyclodextrins, and has led to the development of techniques for binding adducts to specific sites on the cyclodextrin molecule.

The Molecular Physiology and Genetics Section is dedicated to the study of the regulation of physiological functions during aging. The studies on age changes in hormone and transmitter action involve adrenergic receptors and are therefore related to the work of the macromolecular chemistry section. The section is also studying age changes in central nervous system responsiveness, behavioral biology, gene expression and the biology of human longevity. It is also involved in determining whether caloric modification increases life-span in primates as well as in previously studied rodents.

Following are some of the highlights of the research in each section:

### **INORGANIC BIOCHEMISTRY SECTION**

#### Mechanism of Fidelity in RNA Synthesis

We briefly recapitulate our mechanism for achieving fidelity in RNA synthesis. It depends on the *E. Coli* RNA polymerase enzyme recognizing "correct" and "incorrect" bases on incoming NTPs, assuming one conformation that places the incoming NTP with the "correct" base in position for its  $\alpha$ -P to bond the terminal OH of the growing RNA chain, and a different conformation that places an "incorrect" NTP into an unfavorable position for such bonding but into a favorable position to contact an NTPase that destroys the NTP. We have obtained further evidence for details of this mechanism.



### NTPase Specificity

Our laboratory was the first to separate the NTPase activity from RNA polymerase, but others had observed that RNA polymerase had NTPase activity that exhibited a specificity for cleaving nucleotides with the wrong base. We have now demonstrated that our NTPase exhibits such specificity, which in turn is tied to fidelity. Thus, ATP, in the presence of Poly (dT) and poly (dC) is cleaved 5% and 60%, respectively, while 90% and 0.1%, respectively, is incorporated into RNA. In the absence of template, the cleavage activity is higher than with poly(dT):20%. The NTPase prevents different "wrong" bases from incorporation into RNA with different rates of success.

### Relative Importance of RNA Polymerase conformation and NTPase in Assuring Fidelity

The fidelity of RNA synthesis is 100 to 1000 times that predicted by base pairing. We find that removal of NTPase, or mutating RNA polymerase (Univ. of Washington) to remove NTPase activity, decreases fidelity only 2-fold. It appears, therefore, that the conformational flexibility of the RNA polymerase accounts for a much greater part of the effect on fidelity than NTPase activity.

### The Role of Zinc

E. Coli RNA polymerase contains two Zn atoms and one Mg. During the year we encountered some difficulties in RNA polymerase preparation that resulted in the loss of a Zn. After much detective work we discovered the reason for the loss, but an interesting result was the fact that the enzyme that had lost the zinc retained virtually full activity. The curious result is explained as follows: Zn is required as a template to produce the necessary protein conformation for an active enzyme; and, once formed this conformation survives the subsequent loss of the zinc. Such an effect is not uncommon in metal coordination chemistry, in which a metal template is frequently used to organize monomeric constituents into a macrocyclic ligand, but the application of this principle in enzyme structure is novel.

### Yeast RNA Polymerase

We have greatly improved the preparation of yeast RNA polymerase II, and have found two atoms of Zn per molecule, in experiments that we will consider preliminary. (The literature contains many incorrect Zn analyses in biological systems, so we must be very careful.) If our results thus far are confirmed, they represent one more similarity between this eukaryotic enzyme and the E. Coli RNA enzyme. We believe that the mechanism for achieving fidelity in E. Coli RNA polymerase may be similar to the mechanism in eukaryotes.

## **IN VIVO NMR UNIT**

### Aging Effects on Exercise

No significant age changes result from data from 26 BLSA volunteers performing a constant isometric exercise protocol including contraction at 30% of the individual's maximum for three minutes. Findings: **pH**: pH decreased from a mean of  $7.04 \pm 0.03$  at rest to  $6.79 \pm 0.13$  ( $p < 0.01$ ) after three minutes of handgrip exercise, and returned to baseline during recovery. There was no systematic variation of pH either at rest or end-exercise as a function of age. **PCr/p<sub>i</sub>**: PCr/p<sub>i</sub> decreased from a mean of  $10.2 \pm 0.96$  at rest to  $1.1 \pm 0.68$  ( $p < 0.01$ ) at the end of the exercise period, and returned to baseline during recovery. Again, there was no systematic variation of PCr/p<sub>i</sub> as a function of age, either at rest or at end-exercise. In addition, the time constant of both initial PCr recovery and full PCr recovery showed no variation with age. These results suggest that age per se has little effect on forearm skeletal muscle metabolism during isometric exercise at a fixed relative workload.





### Saturation Transfer Studies

These have been undertaken in an attempt to significantly improve the accuracy of kinetic measurements. We have found that experiments with spillover and incomplete saturation can be modeled by a modification of the Bloch equations used to describe transient saturation transfer. We have demonstrated that these experimental effects, when present to a degree commonly seen in in-vivo experiments, can lead to substantial errors in derived reaction rates and spin-lattice relaxation rates. We can therefore make improved measurements by using a more appropriate mathematical model.

### Estimation of spin-lattice relaxation times ( $T_1$ 's)

This value is important throughout in-vivo NMR spectroscopy. We have developed a theory for the optimal design of inversion recovery experiments for measuring values of  $T_1$  using a two-stage design. We have demonstrated that an efficient strategy for these experiments is to make a preliminary estimate of the  $T_1$  in the first stage, followed by a second stage of refinement. In certain instances, this two-stage methodology yields results which are substantially more accurate than a single-stage experiment over a wide range of  $T_1$  values, as well as resulting in a considerable decrease in measurement time.

### Improving the Speed and Accuracy of Kinetic Measurements

Such measurement is of crucial concern in in-vivo NMR, for reasons of comfort in human subjects, and stability in organ preparations. The design of a kinetic magnetization transfer experiments is largely defined by the choice of measurement time points, usually made arbitrarily. However, we have shown that the choice of time points can have a significant influence on the accuracy of these measurements, and developed a rigorous algorithm based on noise analysis which enables the experimenter to select these times in an optimal fashion.

### Amyloid in Alzheimer's Disease

We have described and published the backbone structure of the nine-amino acid amyloid fragment discussed above to a resolution of approximately 0.2Å. We find that a cis peptide bond is present between residues corresponding to gly-37 and gly-38 in  $\beta$  amyloid, which may have an important role in the stability of amyloid plaques.

## **MACROMOLECULAR CHEMISTRY SECTION**

### Chemistry of Cyclodextrins

Hydroxypropyl cyclodextrin is an amorphous mixture of great number of hydroxypropyl ethers of cyclodextrin. The interactions occurring when such mixture dissolves a lipophile are best evaluated when individual and crystalline components of this mixture or their congeners are prepared and studied. Using this approach and enjoying collaboration with a noted crystallographer, we established that whenever possible the hydroxypropyl or another substituent is inserted into the cavity of another cyclodextrin molecule - i.e. the substituent competes with the lipophile to be solubilized; fortunately this competition is rather weak. Furthermore we established that the cavity of cyclodextrin favors (S) enantiomers over their (R) antipodes.

### Biology of Cyclodextrin

By collaborative efforts we established that some of the untoward effects of large doses of hydroxypropyl cyclodextrin administered orally to animals probably stem from the solubilization and consequent easy absorption of lipophile toxins and carcinogens routinely present in food. There are several critical medical uses of hydroxypropyl cyclodextrins (e.g. treatment of mycotic infections of gastrointestinal tract) and non medical uses (e.g. stabilization of the sweetener aspartamate in soft drinks) where hydroxypropyl cyclodextrin may enter the body in large amounts and the above findings have direct health relevance.



## **MOLECULAR DYNAMICS SECTION**

### **Hypoxic Erythrocyte Damage Decreases Deformability**

Oxygen delivery to the tissues requires that the erythrocyte deform in order to pass through the narrow pores in the capillary bed. In order to evaluate the possible relevance of hypoxic damage to oxygen delivery, changes in deformability were determined after incubation at different partial pressures of oxygen. The results indicate decreased deformability at an intermediate  $pO_2$  which coincides with increased superoxide production.

### **Decreased Erythrocyte Deformability in Old Rats**

Three different measures of erythrocyte deformability were used to evaluate changes in deformability during animal aging; 1) The ratio of the discoid volume in PBS relative to that of the swollen volume in half-diluted PBS was used as a measure of excess surface area required for the cell to deform and pass through narrow pores; 2) The mean transit time for cells to pass through a 5 $\mu$  pore; and 3) The relative fraction of a class of slowly deforming cells which could limit the rate of blood flow through the capillary bed. All three parameters indicated a loss in deformability during aging. These results suggest that alterations in erythrocyte function may contribute to the pathophysiology of aging.

### **The Relationship Between Deformability and Function**

Having shown that aging influences erythrocyte deformability, we are attempting to determine to what extent these changes in deformability can influence function. We have therefore initiated (in collaboration with D. Danon and D. Ingram) studies designed to alter erythrocyte deformability in vivo and then to determine how these changes influence function. We have been able to decrease erythrocyte deformability in young rats by erythropoietin injections. The erythropoietin causes a stressed erythropoiesis with large cells which become less deformable during circulation. Preliminary studies indicate more errors in learning a maze for rats with less deformable cells.

## **MOLECULAR PHYSIOLOGY & GENETICS SECTION**

### **G-protein Mediated Signal Transduction and Aging**

We continue to elucidate the mechanisms by which certain types of G-protein mediated signal transduction are impaired during aging as a consequence of receptor coupling/uncoupling dysfunctions. Such aging effects on alpha-adrenergic signal transduction appear to be mimicked by saponin and hydrogen peroxide treatment of rat parotid cell aggregates, while inhibition by methanol is secondary to impaired ligand binding to receptors. Preliminary results suggest that S-adenosyl methionine treatment improves alpha-adrenergic stimulated  $IP_3$  production in aged parotid cells, while feeding rats diets enriched with cholesterol or low in saturated fats inhibits stimulated calcium mobilization in cells of young rats. Most recently, a cell free parotid membrane preparation has been shown to exhibit the same age related changes in stimulated  $IP_3$  production documented in intact cells.

### **The Striatum, Hippocampus and Second Messenger Function in Senescence**

Earlier findings indicated that deficits in muscarinic enhancement in K<sup>+</sup>evoked DA release (K<sup>+</sup>ERDA) in the striatal slice appear to be the result of specific alterations early in muscarinic ST. Current findings suggest that these declines are the result of specific, structural (possibly free radical induced) changes in the membranes which compromise muscarinic receptor (mAChR)-G protein interactions. This research has shown that the incubation of striatal slices from senescent animals with the fluidizing and methylating agent, S-adenosyl-methionine, (SAM) will restore oxotremorine-enhanced K<sup>+</sup>ERDA, while incubation of



striatal slices from young animals with cholesterol will significantly decrease oxotremorine-enhanced K<sup>+</sup>ERDA, and that membrane viscosity increases as a function of age. It has also shown that there may be impaired coupling/uncoupling of mAChR from their respective G proteins during aging in both the hippocampi and striata in both aged rats and in rats (as assessed via low K<sub>m</sub> GTPase activity) exposed to <sup>56</sup>Fe heavy particle irradiation. It can also be restored in aged rats by pre-incubating the tissue in SAM.

We have also observed age-related deficits in carbachol-stimulated low K<sub>m</sub> GTPase in basal ganglia and hippocampi of aged human subjects. Similar, more pronounced, deficits have been observed in basal ganglia and hippocampi from Alzheimer's victims. However some restoration of this activity can be achieved with SAM.

In addition, we have shown that senescent male F344 rats fed high cholesterol (4.5% cholesterol), high saturated fat (7.5% coconut oil), or low fat (1.4% soybean oil) diets for one month show exaggerated deficits in muscarinic stimulated K<sup>+</sup>ERDA. In mature (13 mos) male F344 rats there is no decrease in stimulated K<sup>+</sup>ERDA, but a reduction in oxotremorine-stimulated low K<sub>m</sub> GTPase in all treatment groups and carbachol-stimulated low K<sub>m</sub> GTPase activity in high cholesterol and low fat groups. Preliminary small angle X-ray diffraction analyses of cortical synaptosomes from mature rats fed a low fat diet show a marked decrease in membrane width.

#### Loss of Striatal D<sub>2</sub> Receptor mRNA in Senescence and Selective Vulnerability to EAA

Previous experiments revealed an age-related 50% loss of D<sub>2</sub> receptors mRNA that was partially the result of cell loss and decreased synthesis. Additional experiments indicated that the decline was the result of specific decreases in the competence of old nuclei for the production of D<sub>2</sub> mRNA. Preliminary results, from in situ hybridization studies using digoxigenin labeled D<sub>2</sub> receptor cDNA, indicate that D<sub>2</sub> mRNA may be preferentially lost from larger cells in the striatum.

A tissue culture model, has revealed selective toxicity of striatal dopamine D<sub>2</sub>-receptor containing neurons to the excitatory amino acid analog kainic acid and DA itself. The loss of cells *in vitro* paralleled the decreases seen *in vivo*, which indicated the potential for further investigation into the mechanisms of the age-related loss of these cells using this tissue culture system. Equipment malfunctions have hampered research in this area.

#### Stability of Proper Gene Expression

Our collaboration with Dr. Arifa Khan of the National Inst. of Allergy and Infectious Diseases, NIA, has continued, in which we have been studying the possible age-dependent expression of endogenous MuLV-related sequences in mouse and endogenous retroviral sequences in human tissues. These retroviral studies indicate that endogenous viral gene expression is not static but rather is highly variable throughout the entire life span of an animal.

#### Evaluation of the Spin-Trap Agent, PBN

Our laboratory has focused on determining if PBN reverses the age-dependent decrease in multicatalytic protease activity. Preliminary results indicate that the protease activity does not increase with age and that PBN may not be effective in increasing proteolytic activity in old animals (rats) to that approaching young animals. Recent evidence indicates also that activity of the protease is dependent on source and age of substrate preparation. We also have evidence indicating that PBN given orally to old mice may increase life span. Other studies have indicated the possibility that PBN may act by a nitric oxide delivery agent to specific neuronal sites and is involved in stimulation of dopamine release in old rats. We now have evidence that PBN does degrade in the presence of ultraviolet light or hydroxyl radicals to form nitric oxide compounds.



### Can Caloric Modification of the Diets of Monkeys Affect Aging Rate?

Monkeys in both the ad libitum and reduced calorie diet groups continue to be in generally good health. The restricted animals in our initial rhesus group continue to exhibit marked reductions in body weight and skeletal length, while those in our second group are only slightly decreased compared to ad libitum fed counterparts. In light of the previously noted differences in cholesterol and HDL<sub>2B</sub>, genetic fingerprinting analyses using lipoprotein gene probes will be initiated shortly to attempt to characterize possible differences between the two populations.

We have confirmed that the diet restriction induced delay in rhesus skeletal maturation, suggested in part by an approximately one year delay in the decrease in circulating alkaline phosphate, is indeed a reflection of the enzyme form characteristic of bone. Nearly 90% of this enzyme in serum is the bone form with about 10% from liver. The corresponding figures for squirrel monkeys are 60 and 40%, respectively. Serum levels of IGF-1, the mediator of growth hormone actions are also reduced in aged rhesus monkeys, analogous to the changes reported for rodents and humans. Preliminary studies have not yet detected differences between diet groups, however. Serum fibroblast proliferation factor activity also appears to decrease with age. This is consistent with an age associated decrease in skin fibroblast clonal efficiency. Although no significant effects of diet on this parameter have been observed to date, a trend toward greater loss of clonal efficiency in young rhesus monkeys of our initial group seems to be emerging. Stimulated lymphocyte calcium mobilization decreases with age in all rhesus groups irrespective of diet. This observation is consistent with the previously noted decrease in lectin stimulated mitogenesis.

We have completed a preliminary estimate of "biological age" utilizing a number of the blood chemistry parameters under study. Results for our initial rhesus group suggest that caloric restriction may be exerting some retardation of aging. However, the analysis is heavily influenced by maturational and early life changes. It will now be necessary to obtain adequate data for post-maturational age changes.

### Drug Development for Cognitive Enhancement

Collaborating with Dr. N. Grieg, we have continued to analyze effects of novel cholinomimetics on learning performance. We tested the effects of a potent anticholinesterase, phenserine, for improving learning performance of aged rats in the 14-unit T-maze. This drug has a long duration of action (>1 hr) and was observed to improve learning at relative low doses (<3mg/kg). In addition, we have found that glycine agonism with milacemide improves performance of young mice in a swim maze. Attempts to combine this treatment with phenserine to further improve performance in aged mice were not successful.

### Glutamate and Nitric Oxide Involvement in Learning

Collaborating with Dr. E. London, we continue to examine the involvement of the glutamatergic system in memory processing. We have now found that compounds delivered centrally that block the activity of nitric oxide synthase (NOS) and thus inhibit production of nitric oxide (NO) which is regulated by glutamate receptor activation will impair maze performance in young rats. This impairment can be partially attenuated by treatment with sodium nitroprusside which is a NO generator. We have perfected a histological method for assessing the number of NOS containing neurons and the density of NOS containing neurites. Using highly specific ligands for different sites on the glutamate receptor, we have also found that the age-related decline is observed primarily in a site located in the cationic channel controlled by the receptor.





### RBC Size and Learning

In collaboration with Drs. D. Danon and J. Rifkind, we have begun to use the synthetic hormone, erythropoietin, to increase the size of erythrocytes (RBC) as a possible model of thrombosis that can occur in the aged brain. We have found that this treatment does increase size and reduce deformability of the RBCs and that these parameters are correlated with maze performance in young rats.

### Laminin and Laminin Receptors in Brain

Collaborating with Drs. M. Jucker, H. Kleinman, and L. Walker, we have continued to examine the immunocytochemical profile of antibodies to a 110 kD laminin binding protein (LBP) with particular focus on identification of unique pathology in the brains of old C57BL/6J(B6) mice. This antibody recognizes granular deposits occurring in several specific brain regions but primarily concentrated in the hippocampus with an age-dependent increase in incidence. Additional immunocytochemical analysis has further confirmed the relationship of these deposits to astrocytes and has suggested a composition that includes laminin and heparin-sulfate proteoglycan, which has been suggested to be a precursor condition for beta-amyloid deposition in Alzheimer's disease. We have examined many other mouse strains to find that incidence of the pathology is restricted to the B6 strain and any F<sub>1</sub> hybrid carrying a maternal B6 background. However, we have found that the senescence accelerated mouse (SAM), which is unrelated to the B6 strain, has the same pathology which appears at an earlier age and with greater intensity than we have observed in the B6 strain. We have found that the incidence of the pathology in hippocampus of aged B6 mice is unrelated to performance in swim maze performance, although marked cognitive impairment has been reported to occur in the SAM strain.

### Adenoviral Vectors for Gene Therapy in Brain

We have initiated a collaboration with Drs. A. Mastrangeli and R. Crystal to determine the feasibility of using adenoviral vectors for delivery of specific genes to brain. Using the lac-Z gene as color marker in brain, we have confirmed that injection of adenovirus into the neuropil will permit neuronal transection and retrograde transport of the vector by axons. In addition, injection of the vector into the lateral ventricle results in a distribution throughout the ventricular system with preferential uptake of the vector into areas of neocortex damaged by a photochemical induced thrombosis.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00044-20 LCMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metal Ions and Information Transfer; mechanism of RNA Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Gunther L. Eichhorn, Chief, LCMB, NIA

Others: James J. Butzow, Commissioned Officer, IBS LCMB NIA

Patricia Clark, Research Chemist, IBS LCMB NIA

Chris Janzen, IRTA Fellow, IBS LCMB, NIA,

COOPERATING UNITS (if any)

Israel Institute for Biological Research (D. Waysbort); Univ. of Western Ontario (S.J. Karlik); MDS (LCMB) (J. Rifkind), Centre for Cellular and Molecular Biology; (D. Chatterji); Univ. of Utah (L. Powers)

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of metal ions on nucleic acids structure and function have been determined. The primary focus now is on the mechanism of RNA synthesis at the active site of RNA polymerase. The geometry of interaction at the active site of the enzyme is being probed during the transcription process to determine how this geometry changes during transcription and affects its mechanism. Evidence has been obtained that the enzyme assures fidelity in copying the genetic code by conformation changes sensitive to complementarity or non-complementarity between DNA base and the base on the incoming nucleoside triphosphate. A comprehensive mechanism of fidelity has been developed that allows a Mg(II) switch to effect or prevent incorporation of the right and wrong substrate, respectively.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AG 0381-03 LOMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Studies of Aging in Cells, Organs, and Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Richard G. S. Spencer, M.D., Ph.D., Unit Chief, In-vivo NMR

Others: Gunther L. Eichhorn, Ph.D., Chief, LOMB, Jerome L. Fleg, M.D., Jack Henningfield, Ph.D., David Spector, Ph.D., Jan Busby, M.D., Mark Blackman, M.D., George Roth, Ph.D., George Weiss, Ph.D., Jim Ferretti, Ph.D.

COOPERATING UNITS (if any)

Cardiovascular Section, Clinical Physiology Branch, NIA

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry, In-vivo NMR Unit

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2

OTHER:

.5

CHECK APPROPRIATE BOXES)

- ☒ (a) Human ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NMR spectroscopy is currently being used at the NIA to study the phosphorus metabolism of peripheral muscle in BLSA subjects and other volunteers, as well as in animals. Age-related and exercise-related effects are under investigation. Methodologic studies to further develop kinetic and spin-lattice relaxation time measurement methodology are also under active investigation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00382-03

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies by Solid-State NMR

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard G. S. Spencer, Unit Chief, In-vivo NMR

Others: Robert Griffin, Ph.D., Peter Lansbury, Ph.D., Malcolm Levitt, Ph.D.

COOPERATING UNITS (If any)

MIT Department of Chemistry, and Francis Bitter National Magnet Laboratory

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry, In-vivo NMR Unit

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have successfully elucidated detailed structural features of a nine-amino acid peptide found in the amyloid protein of Alzheimer's disease. More basic work has included development of what is essentially a CPMAS version of TOCSY. We call this experiment DICSY, for dipolar correlation spectroscopy. Other ongoing research includes the theory and demonstration of CPMAS experiments i) to create rotational resonances at scaled chemical shift offsets ii) to explore dipolar interactions involving three nuclei and iii) to selectively invert one sideband manifold overlapping that of another nucleus.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00046-23 LCMB

PERIOD COVERED

October 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Recognition of Lipids and Lipophiles by Cyclodextrin Derivatives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Josef Pitha, Chief, Macromolecular Chemistry Section, NIA/LCMB

Others: Jindrich Jindrich, Ph.D. - Oct. 92 MCS/LCMB/NIA

Jiri Horsky, Ph.D. - March 93 MCS/LCMB/NIA

Bengt Lindberg, Ph.D.

Kazuaki Harata, Ph.D.

Kaneto Uekama, Ph.D.

Alessandro Olivi, M.D. and Andrea Gerloczy, Ph.D.

COOPERATING UNITS (if any)

Univ. of Stockholm, Instit. of Bioscience & Human Technology, Kumamoto Univ., John Hopkins Hospital, Cyclolab, Budapest

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Macromolecular Chemistry Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hydroxypropyl cyclodextrins, chemical derivatives of glucose oligosaccharides, which were originated in the section, form water soluble complexes with lipophiles. Since these derivatives are also non-toxic they have been used as drug solubilizers with enough of success to qualify them for consideration to be included in the next edition of US Pharmacopeia/National Formulary. The present chemical results point out the structural elements which are critical for the acceptable performance of hydroxypropyl cyclodextrins in biomedical applications. The present biological results point to a possible health hazards which may result from the ingestion of very large doses of hydroxypropyl cyclodextrins.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00047-23

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure-Function Relationships in Hemoglobin and Erythrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joseph M. Rifkind, Chief, MDS, MDS LCMB NIA

Others: Omofe Abugo, Visiting Fellow, MDS/LCMB/NIA; Jane Heim, Chemist; P.T. Manoharan; V.S. Sharma; V. MacDonald; D. Danon; D. Ingram; R. Rao

COOPERATING UNITS (if any)

Indian Inst. of Tech., Madras, India; U. CA-San Diego, La Jolla, CA; Letterman Army Inst. of Res., San Francisco, CA; Waldenberg Center for Gerontological Studies; NIA/LCMB/MPGS; U. of Maryland, Baltimore, MD

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Dynamics Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

This project focuses on the mechanism involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. Emphasis is placed on ways in which these functions are impaired and change with age. These studies have focused on the oxidation of hemoglobin, which produces nonfunctional hemoglobin and the simultaneous release of oxyradicals. The enhancement of these oxidative processes under hypoxic conditions is being explored as a possible source of tissue and organ damage, which would be exacerbated during aging. Studies are also included which are directed at the stability of the entire erythrocyte and the erythrocyte membrane.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00301-10 LCMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: I. Hormone Action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G.S. Roth, Ph.D., Chief, Molecular Physiology & Genetics Section, LCMB, NIA

Others: B. Baum, A. Ishigami, A. Richardson, M.A. Kowatch, G. Kokkonen

COOPERATING UNITS (If any)

Patient Care Branch National Institute of Dental Research; V.A. G.R.E.C.C.,  
University of Texas, San Antonio

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

3.3

PROFESSIONAL:

1.3

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is mainly involved in elucidating those mechanisms by which the ability of hormones and neurotransmitters to regulate physiological functions is altered during aging.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00306-5 LCMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reg. of Physiological Functions During Aging:II. Neurotransmitter Responsiveness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: J.A. Joseph, Research Pharmacologist, MPGS, LCMB, NIA  
G.S. Roth, Chief, MPGS, LCMB, NIA

Others: M. Anson, R. Cutler, Ph.D., L. Zhang, Ph.D., D.K. Ingram, Ph.D., P. Mason, Ph.D., Jeremiah Kelly, M.D., Steve Erat

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project attempts to understand those mechanisms involved in age related changes in central nervous system responsiveness.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00303-9 LCMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: IV. Genes and Longevity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. G. Cutler, Ph.D., Research Chemist, LCMB, NIA

Others: D.K. Ingram, G.S. Roth, A. Ayala, A.S. Khan, G. Cao, K. Kitani, H. Alessio, I. Zs.-Nagy.

COOPERATING UNITS (If any)

Miami Univ., Ohio: LMM, NIAID, Bethesda, Tokyo Metrop. Inst. Gerontol.; Verzar Inst. Exp. Geront.

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our research has focused on assessing the possible role oxidative stress may have as an initiator of aging and on mechanisms reducing oxidative stress as possible determinants of longevity. Methods to detect and measure oxidative damage are essential to these studies. We have developed a new assay to measure total oxygen radical absorption capacity (ORAC) of serum. Using this assay, we have found that the ORAC value of serum increases in direct proportion to life span of different mammalian species but appears not to change with age. The spin-trap agent, N-tert-butyl- $\alpha$ -phenylnitron (PBN) has been reported to reverse a number of age-related effects, including a decrease in oxidative stress state. Preliminary results in our laboratory indicate that PBN may stimulate molecular renewal rate in old animals and possibly extend life span. We have not been able to detect any age related changes in alkaline protease activity nor its stimulation by PBN. However PBN when given orally to mice does have some effect in increasing mean life span. PBN and other related spin traps were found to release nitric oxide in vitro and stimulate guanylate cyclase activity after reacting with a free radical. Thus the mechanisms of PBN action may involve delivery of nitric oxide to specific neurological sites in the brain.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00304-7 LCMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: V. Assessment of Primate

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: G.S. Roth, Ph.D., Chief, Molecular Physiology & Genetics Section, LCMB, NIA  
D.K. Ingram, Ph.D., Research Psychologist, MPGS, LCMB, NIA  
R.G. Cutler, Ph.D., Research Chemist, MPGS, LCMB, NIA  
M.A. Lane, Ph.D., IRTA Fellow, MPGS, LCMB, NIA  
Others: J. Knapka, D. Barnard, R. Weindruch, W. Ershler, D. Danon, B. Flynn, L. Olsen, N. Wolf, P. Rabinovitch, C. Harley, M. Reynolds, V. Monnier, S. Ball, D. Cocchi, A. Reznick, H. Kondo

COOPERATING UNITS (if any)

Department of Medicine, University of Wisconsin, Madison, WI, Department of Pathology, University of Washington.

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project is attempting to determine whether caloric modification of the diets of Rhesus and squirrel monkeys can affect aging rate as assessed by various physiological, biochemical and behavioral indices.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00302-10 LCMB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: III. Behavioral Biology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Donald K. Ingram, Ph.D., Research Psychologist, MPGS, LCMB, NIA

Others: E. Bresnahan, D. Danon, R. Crystal, R. Fanelli, J. Finkelstein, J. Flood, N. Grieg, H. Ikari, J. Hengemihle, M. Jucker, H. Kleinman, H. Kuo, E. London, J. Rifkind, G. Roth, A. Shimada, E. Spangler, L. Walker, A. Mastranjeli.

COOPERATING UNITS (if any)

Cornell Univ. Sch. of Med; Essex Community College; Nat'l Inst. Dental Res; JHU Medical School; Nat'l Inst. Drug Abuse; Towson St. Univ.; Miles Pharmaceuticals; Washington Univ. Sch. of Medicine; Weizmann Inst.

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

5.3

PROFESSIONAL:

4.0

OTHER:

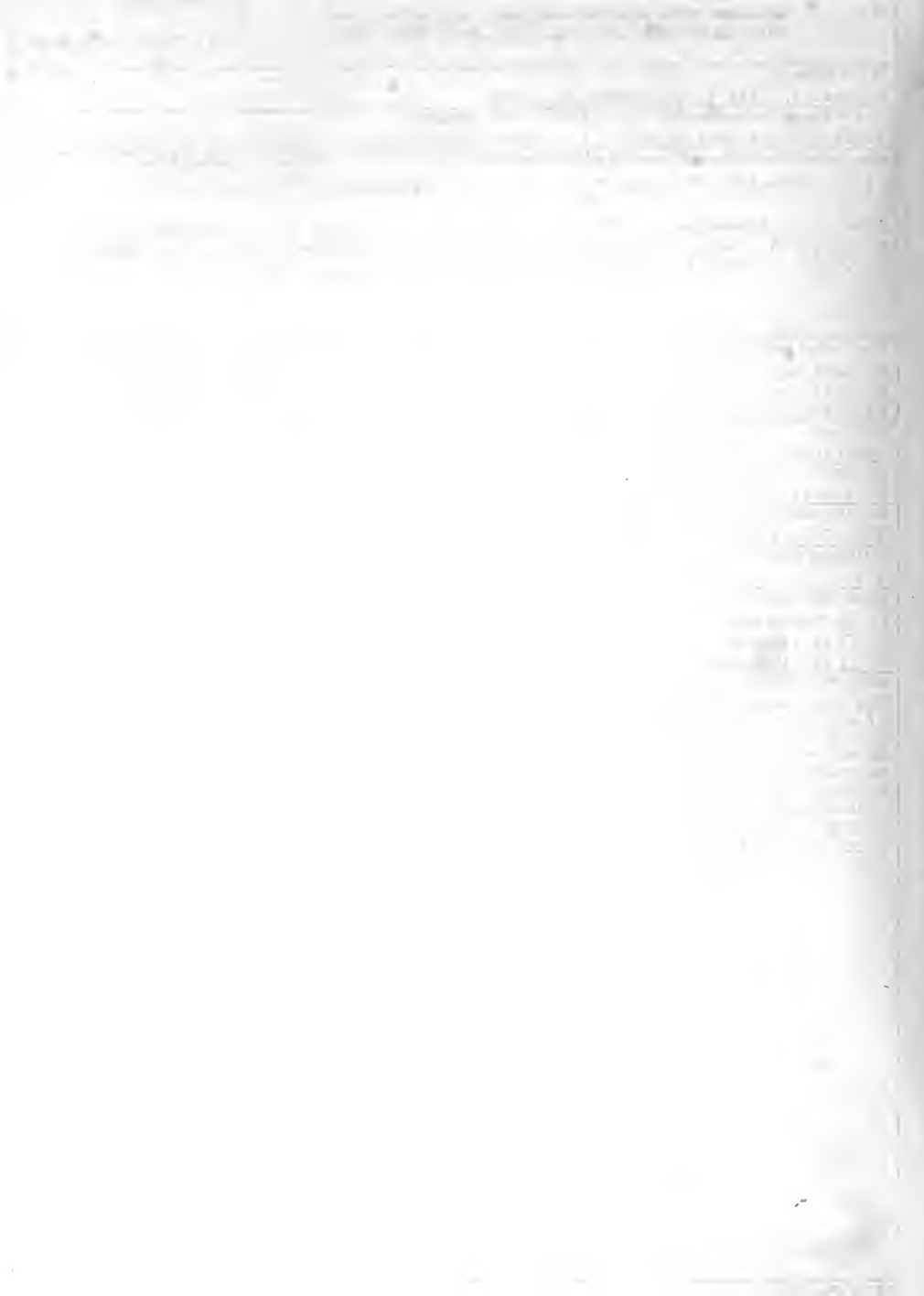
1.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The purpose of this project is to assess the effects of aging at a behavioral level of analysis, to identify neurobiological mechanisms associated with these effects, and to test interventions which might alter age-related performance decrements. Rodent models are tested in a battery of sensorimotor and learning/memory tasks. Neurochemical and neurohistological assays are conducted to determine neurobiological correlates of functional losses. Interventions include dietary restriction, exercise, various pharmacologic treatments and neurotrophic factors. Multiple genotypes are examined to determine possible genetic involvement in the pattern of age-related behavioral impairment.



LN

EDBP

LCP

IMG

LPC





## Summary: Laboratory of Clinical Physiology

LCP is composed of Four Sections and One Unit. All five operate very independently and therefore the Laboratory Summary is presented by individual Section.

### Metabolism Section, LCP -- Annual Report 1993

#### Summary

Research in the Metabolism Section involves human clinical investigation. The subjects are primarily participants in the Baltimore Longitudinal Study of Aging. The comprehensive multidisciplinary periodic evaluation provides unique data for correlative studies among metabolic variables as well as analyses for risk factors for the important chronic illnesses of aging in men and women across the entire age span of years. The primary areas of interest have been (1) glucose and insulin metabolism, (2) body composition including obesity, fat distribution, and lean body mass, (3) plasma lipids and lipoproteins, (4) dietary intake and use of dietary supplements, and (5) development of such end-points as mortality, coronary heart disease, and diabetes mellitus. Some of the findings in this report are: (1) The diagnostic cutpoints for interpreting the glucose tolerance test need to be age-specific; the "impaired glucose tolerance" range of two-hour glucose levels is much too high in older individuals; (2) glucose tolerance in women is sensitive to exogenous hormonal use but not to endogenous fluctuations during the menstrual cycle; oral contraceptive agents are associated with poorer tolerance while post-menopausal estrogen use is associated with improved tolerance; (3) the sagittal diameter of the mid-abdominal area (the anteroposterior diameter) has been shown to be a strong correlate of intra-abdominal fat deposition, the fat depos that is strongly associated with many of the risk factors for the development of diabetes and coronary heart disease ("Syndrome X"). Results from the BLSA population show that the sagittal diameter is predictive of all-cause mortality and of coronary heart disease in younger (less than 55 yr) but not in older men, and this effect is independent of the degree of total obesity. This study also shows strong correlations with risk factors for disease in both men and women, more strongly in younger than in older individuals; (4) the respiratory quotient (RQ) under basal conditions predicted the future development of weight gain with aging. Those men with RQs greater than 0.85 ("carbohydrate-burners") were more likely to gain those with RQs lower than 0.76, indicative of dominant use of fat as a fuel under basal conditions. Thus there are metabolic differences in those who do and to not tend to gain weight; (5) A critical review of published reports from many populations, including the



BLSA, shows a disturbing fact: in community-dwelling men and women, mortality tends to be lowest in those who gain a small to moderate amount of weight as they age while those who lose weight have higher mortality; (6) Meticulous dietary data collection over a period of some 30 years in men and 15 years in women is revealing a number of interesting health implications: (a) Low intake of several B vitamins has been proposed as being possibly related to the development of cognitive decline with aging. Among these, intake of B6 was quite low in this population. But, when the Benton Visual Retention Test was used as the measure of cognitive decline, only low calorie and low riboflavin intake were significantly related to decline; (b) state-of-the-art photography of the lens of the eye showed that nuclear cataracts were associated with low plasma alpha-tocopherol levels while cortical cataracts were associated with low retinol levels; (c) intake of minerals showed a surprisingly large percentage of deficient individuals as judged by RDA standards: Calcium intake was generally much below the 1500 mg/d intake recommended for post-menopausal women; iron intake was low in 25% of premenopausal women but only in 10% in post-menopausal women; the median magnesium intake was below RDA standards and some 40% of men and women were deficient in zinc intake. It is of importance that the BLSA participants are upper-middle class, highly educated, health conscious individuals. Nevertheless, apparent nutritional deficits are common.

1870-1871

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00216-03 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alterations in Carbohydrate Metabolism in Normal Aging and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Reubin Andres, M.D. Chief, Metabolism Section, LCP

Others: Denis C. Muller, M.S. Computer Programmer/Analyst, LCP  
Jordan D. Tobin, M.D. Chief, Applied Physiology Section, LCP

COOPERATING UNITS (If any)

Drs. Gail Cherry-Peppers, Bruce J. Baum, and Jonathan A. Ship., NIDR  
Drs. Susan Vitale, Sheila West, and Hugh R. Taylor, Wilmer Eye Institute,  
The Johns Hopkins University

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Metabolism Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH< Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.6

PROFESSIONAL:

0.8

OTHER:

2.8

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Diabetes is a common disease of the older population of the United States. Estimates of the prevalence of this disease from the National Health Interview Survey indicate that about 2.4% of the U.S. population, approximately 5.5 million Americans, consider themselves to have diabetes. It is estimated that for every known case of diabetes, there is an unrecognized case. In addition to definite diabetes, impaired glucose tolerance, a state of borderline glucose metabolism, exists. Diabetes is a known risk-factor for microvascular (renal, retinal, and neurologic) disease and macrovascular disease (accelerated atherosclerosis). The effect of these morbid conditions on American health care expenses is enormous. In an effort to identify risk factors for the development of diabetes, and identify potential areas for intervention, 30 years of longitudinal data from the Baltimore Longitudinal Study of Aging are being examined. We previously reported that fasting glucose values have a graded effect on the rate of development of overt diabetes. The borderline zone of 115-139 mg/dl is artificial. Increased incidence of diabetes can be demonstrated in subjects aged 28-59 yr with fasting glucose levels as low as 103-107 mg/dl. We now have examined the predictive power of the two hour value on a standardized oral glucose tolerance test. Values of 140-199 mg/dl have been labeled as "impaired" glucose tolerance, the implication being that risks of future development of diabetes and its complications are increased. These values were found to be appropriate for younger adults (28-59 yr) but are set too low for adults aged 60 and over. No cases of diabetes developed in men whose two-hour glucose value was below 157 and no statistically significant increase in development of overt diabetes occurred until levels of 200 or more mg/dl were reached. Thus standards need to age-specific; they are set too low for older adults.

Female sex hormone status was evaluated as a potential modifier of glucose tolerance. No effect of the phase of the menstrual cycle was found, but women on oral contraceptive therapy had significantly poorer tolerance than those not on treatment. In contrast, post-menopausal women on estrogen replacement had better tolerance than those older women not on replacement.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00217-03 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Insulin Secretion and Insulin Sensitivity: Blood Pressure, Age, Obesity, and Sex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Denis C. Muller, M.S. Computer Programmer/Analyst, LCP  
Reubin Andres, M.D. Chief, Metabolism Section, LCP

Other: Jordan D. Tobin, M.D. Chief, Applied Physiology Section, LCP

COOPERATING UNITS (if any)

Dariusz Elahi, Ph.D., Division of Gerontology, Beth Israel Hospital, Boston, MA

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Metabolism Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Subjects were participants in the Baltimore Longitudinal Study of Aging. Plasma samples were obtained during an oral glucose tolerance test and insulin was measured by radioimmunoassay. Cross-sectional and longitudinal analyses were employed to gain insight into the complex inter-relationships among post-load and fasting plasma insulin levels and age, sex, obesity, fat distribution, lipids, and blood pressure. Due to the increased morbidity associated with hyperlipidemia, hypertension, hyperinsulinemia and obesity, the projects were directed toward attempting to determine the causal order, if one exists, among these metabolic variables as well as the roles of sex and aging. From the cross-sectional findings of this project it can be concluded that: (1) Correlations of blood pressure with plasma insulin levels after adjustments for age, obesity, and fat distribution were entirely non-significant. 2) Sex differences in fasting and post-load insulin levels are explained by differences in body habitus but insulin levels decline with age per se.

In order to evaluate the potential role of insulin insensitivity as a cause of the glucose (G) intolerance of aging, we performed 230 hyperglycemic clamps, 85 on young (Y, 24 to 39 years), 47 on middle age (M, 40 to 59 years), and 98 on old (O, 60 to 90 years) carefully screened subjects of the Baltimore Longitudinal Study of Aging. The two hour plasma G levels on an oral glucose tolerance test were less than 140 mg/dl in Y and M and 180 mg/dl in O. The old group was further dichotomized at 140 mg/dl into a "normal" group (ON) and an "impaired" group (OI). Four hyperglycemic plateaus were created: 3.0, 5.4, 7.9 and 12.8 mmol/L above basal. Three measures of glucose tolerance were derived: (1) G at 2 hours after glucose ingestion, (2) glucose utilization, M, at each hyperglycemic plateau, and (3) glucose decay constant, K, obtained at the conclusion of each clamp. These showed that the young group had the best glucose tolerance (Y:M=ON:OI). Despite these age differences in glucose tolerance, both the early and late phase plasma insulin responses during the clamp were remarkably similar among the groups. In contrast, insulin-dependent glucose uptake, a measure of tissue sensitivity to insulin, was decreased in the old-impaired group at every plateau. We conclude that healthy, active older subjects showed moderate intolerance to oral and IV glucose and that the major mechanism of this physiological aging process is decreased insulin sensitivity.





DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00218-03 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Respiratory Quotient and Metabolic Rate as Predictors of Major Weight Gain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Reubin Andres, M.D. Chief, Metabolism Section, LCP

Other: Denis C. Muller, M.S. Computer Programmer/Analyst, LCP

COOPERATING UNITS (if any)

John D. Sorkin, M.D., Epidemiology Fellow, EDB, NIA  
Jacob C. Seidell, Ph.D., National Institute of Public Health and Environmental  
Protection, Bilthoven, The Netherlands

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Metabolism Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has been observed that reduced metabolic rates and high 24-hour respiratory quotients are predictors of subsequent weight gain in Pima Indians. It is unknown whether or not this is true for other populations and whether the fasting RQ is a predictor of weight gain as well. Seven-hundred and seventy-five men (aged 18-98 years) participating in the Baltimore Longitudinal Study of Aging were followed for an average of 10 years. Basal metabolic rate and RQ were measured on their first visit and related to subsequent weight change. Deviations from the predicted value of basal metabolic rates (predicted from their estimated fat free mass) were calculated. Average weight change was 0.1 kg (s.d. 6.4 kg); 122 (15%) gained more than 5 kg and 40 (5%) more than 10 kg during the follow-up. After adjustment for initial age, body mass index, fat free mass, and duration of follow-up, RQ, but not BMR or deviations from predicted BMR, was positively related to weight change ( $p < 0.001$ ). Major weight gain (5-15 kg) was related to initial RQ in non-obese men only (body mass index  $< 25 \text{ kg/m}^2$ ). From Cox proportional hazard regression analyses the adjusted relative risk of gaining 5 kg or more in initially non-obese men with a fasting RQ of 0.85 or more was calculated to be 2.4 (95% confidence interval: 1.1-5.3) compared to men with a fasting RQ less than 0.76. Conclusion: A relatively high fasting RQ is a weak but significant predictor of substantial weight gain in non-obese white men.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00219-01 LCP

PERIOD COVERED

October 1, 1992, to September 20, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Long-term Effects of Change in Body Weight on Mortality

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Reubin Andres, M.D. Chief, Metabolism Section, LCP

Other: Denis C. Muller, M.S. Computer Programmer/Analyst, LCP

COOPERATING UNITS (if any)

John D. Sorkin, M.D., Epidemiology Fellow, EDB, NIA

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Metabolism Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objective of this project was to summarize published studies that analyze the effects of long-term change in body weight on all-cause mortality. The thirteen reports are from eleven diverse population studies, seven from the United States (including the Baltimore Longitudinal Study of Aging) and four from Europe. All studies included a weight change period of four years or more followed by a mortality assessment period of eight years or more. Changes in weight occurred during the adult years of life, the youngest starting age being 17 years. Study details varied widely in terms of numbers of subjects and deaths, ages at initial and final weight measurements, duration of the mortality follow-up period, consideration of cigarette smoking and other potential confounders, exclusion criteria, temporal separation between the weight change and mortality follow-up periods, and the method of assessment of the association of weight change and all-cause mortality.

**Conclusions.** Despite the diversity of (a) populations studied, (b) degree of "clinical clean-up" at entry, (c) techniques for assessing weight change, and (d) differences in analytical techniques, including consideration of potentially confounding variables, certain conclusions may be drawn. The preponderance of the evidence is that during the adult years, highest mortality occurs in those who either have lost weight or gained excessive weight and lowest mortality is generally associated with modest gain in weight.

These results support the use of age-specific weight-for-height tables, as recommended initially by the Gerontology Research Center, and as supported subsequently by the 1989 National Research Council publication Diet and Health and the 1990 Dietary Guidelines for Americans (USDA and USDHHS).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00220-01 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Body Composition and Fat Distribution in Aging and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Reubin Andres, M.D. Chief, Metabolism Section, LCP

Other: Denis C. Muller, M.S. Computer Programmer/Analyst, LCP

COOPERATING UNITS (if any)

John D. Sorkin, M.D. Epidemiology Fellow, EDB, NIA  
Jacob C. Seidell, Ph.D., National Institute of Public Health and Environmental  
Protection, Bilthoven, The Netherlands

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Metabolism Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Anthropometry and cardiovascular risk factors were measured in men and women of the Baltimore Longitudinal Study of Aging. Cross-sectional and longitudinal statistical analyses examined the relationships among body habitus (body composition and fat distribution) and aging, mortality, and selected cardiovascular risk factors (blood pressure, glucose tolerance, lipid levels). The abdominal sagittal diameter is a simple anthropometric measurement that can be used in population studies. Its use as a surrogate for intra-abdominal fat measurement (by CT or MRI) was suggested from studies conducted by Gothenburg, Sweden scientists. It was therefore used as an index of an individual's central adiposity. SHR was significantly correlated to the risk factors in both younger and older men and women. However the correlations were considerably stronger in the younger subjects. Increasing abdominal sagittal diameter was associated with increased all-cause mortality and cardiovascular mortality in younger BLSA men (<55 years) but not older men (adjusted for age, height and body mass index). From the findings of this project, it can be concluded that: (1) the abdominal sagittal diabetes is positively correlated to cardiovascular risk factors in men and women; (2) the strength of this relationship decreases with age; (3) and the abdominal sagittal diameter is a strong predictor of mortality in younger adult men, independent of the degree of obesity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00901-01 LCP

PERIOD COVERED

October 1, 1992, to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dietary intake, supplemental intake, & plasma levels of vitamins in men & women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Denis C. Muller, M.S. Computer Programmer/Analyst, LCP

Others: Reubin Andres, M.D. Chief, MS, LCP

COOPERATING UNITS (if any)

Dept. Psychology, American University, Washington, DC  
Marjorie Thompson, Graduate Student

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Metabolism Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nutritional evaluation in the male and female participants of the Baltimore Longitudinal Study of Aging (BLSA) has been carried out by periodic collection and analysis of 7-day dietary diaries and by measurement of vitamin levels in plasma. The diary technique has provided data over a 30 year period in men and a 10 year period in women. The ages of the participants range from 20 to 95 years. Since the BLSA is a multi-disciplinary study it is possible to carry out correlations between nutritional intakes and levels and other potentially related variables and outcomes.

Conclusions - Many analytical studies are currently in progress. Some of the results of recent analyses are presented here. It is of interest that a high percentage of this middle to upper-middle class highly educated population take nutritional supplements, but those who do have less need for them than those who do not; that is, the quality of the diet intake is better in those who take supplements. Among the B vitamins that have been proposed as being potentially related to cognitive decline, B6 intake is the poorest in this population, followed by folacin, with B12 being the least deficient. Cognitive function decline in this analysis was evaluated from repeated administration of the Benton Visual Retention Test. Decline was significantly related only to relatively low total caloric and riboflavin intake.

Anti-oxidant vitamins (alpha-tocopherol and retinol) were examined with respect to development of nuclear and cortical cataracts as determined by modern photographic methods. Since knowledge of the presence of a cataract could lead to a change in vitamin intake, vitamins status was determined at least 2 years prior to the photography. Plasma alpha-tocopherol level was significantly related to the presence of nuclear cataract while plasma retinol level was related to cortical cataract.

Plasma ascorbic acid level was significantly related to the high density cholesterol (HDL) level as well as to the HDL-2 level. Supplementary C intake led to higher plasma levels only up to a threshold of 250-300 mg intake per day. Above this intake no further increase in plasma level occurred.





## DIABETES UNIT, LCP, ANNUAL REPORT 1993

### Summary

Diabetes Mellitus is a major illness among the elderly, markedly increasing both mortality and morbidity. The present methods for treating diabetes yield only mediocre results. The work of this Unit continues to be directed to the design of new treatments for diabetes mellitus in the elderly, aimed at (1) restoring early insulin secretion, (2) normalizing insulin action at the target cells, and (3) slowing the age related deterioration in insulin secretion.

In each of these areas, the research programs strives to (1) conduct the search for therapy in the context of cutting edge scientific inquiry; (2) use a "molecular" approach in the broadest sense, i.e. selecting in each case a specific molecule as the target site for the therapeutic intervention; (3) have available for each area appropriate human populations; non-human primate and rodent models; fresh cells and cell lines; protein chemistry and molecular biology; and (4) develop links early to pharmaceutical firms that share our interests and whose talents complement those in the Unit.

**Restoring Early Insulin Secretion --** Our recent studies have shown that incretin hormones, namely GLP and GIP, synergize with glucose to stimulate insulin secretion, and also stimulate gene expression of proteins involved in the insulin secretory pathway. Using the cloned rat GLP receptor, we have shown [using PCR techniques] that these receptors are present not only in insulin producing cells of the endocrine pancreas but also in brain, muscle and fat. To determine whether this finding has physiological relevance, we have instituted metabolic assays and have shown that GLP increases glucose utilization in 3T3-L1 adipocytes. An investigation of the trophic effect of GLP in beta cells continues as well a more extensive investigation of the extrapancreatic effects of GLP. We have also been examining the potential role of nitric oxide as a mediator of insulin release and the possible modulation of glycogen synthesis by amino acids.

**Insulin Action at the Target Cells --** These projects relate to the identification of a putative insulin receptor phosphatase. We have demonstrated that there is specific interaction between the insulin receptor and membrane-associated protein tyrosine phosphatase(s) in two *in-vitro* systems. We are making progress in identifying the specific enzyme and have also prepared peptides that are potent and specific inhibitors.



Retarding Age Related Deterioration -- Recent studies have shown that part of the loss of glucose tolerance that occurs with aging is due to secretory abnormalities of the B-cell of the pancreas. We find that not as many cells release insulin with age and the amount of insulin secreted per cell is decreased. In conjunction with this, the messenger RNA for insulin also decreases with age, more than other islet messages. There is also a decline in REG gene expression with normal aging. This project will continue to tease out the trophic factors responsible for maintaining normal B-cell and islet function, and look for agents that may modify the seemingly inevitable decline in function over time.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00213-03 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tyrosine phosphatases and insulin resistance in the aged

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Bernier Senior Staff Fellow LCP, NIA

Others:

A.S. Liotta	Special Expert	LCP, NIA
H.K. Kole	Senior Staff Fellow	LCP, NIA
D.D. Shock	Biologist	LCP, NIA

COOPERATING UNITS (if any)

Jesse Roth, Director, Division of Geriatric Medicine and Gerontology,  
Johns Hopkins University School of Medicine  
L. Adams, Lab Technician, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Insulin resistance at the target cell is common in non-insulin dependent diabetes mellitus of the elderly. We have been investigating novel ways to identify and characterize a putative insulin receptor phosphatase in an attempt to understand how this phosphatase activity can be regulated *in vivo* as a target for treatment.

We have demonstrated that our recently introduced permeabilized cell model offers unique opportunities to investigate the intimate regulation of insulin receptor phosphorylation and dephosphorylation by closely-associated membrane-bound protein tyrosine phosphatase(s). It is suggested that compartmentalization within the cell and interactions with other putative regulatory proteins are maintained. Furthermore, this system has allowed the study of the activity of normal and mutant insulin receptors and the effect of synthetic peptides on the extent and pattern of receptor phosphorylation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00214-03 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Use of an in vivo model system to investigate NIDDM

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.M. Egan Senior Staff Fellow LCP, NIA

Others:

R. Perfetti	Visiting Associate	LCP, NIA
T. Henderson	Science Lab Tech	LCP, NIA
A.S. Liotta	Special Expert	LCP, NIA
C. Montrose	Staff Fellow	LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine  
L. Adams, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Age is an independent risk factor for NIDDM. Age is also associated with a decline in insulin secretion. Using  $\delta$ -cells from Wistar rats from the colony of aged rats at the GRC, we showed that insulin release in response to glucose decreases with age of the donor. This is a result of fewer beta cells releasing insulin as well as less insulin released per cell. We found that mRNA for insulin is preferentially diminished in islets, with glucokinase and glucagon messages unaffected. Therefore, it appears that throughout the lifespan of the rat, insulin message is decreasing. Islets compensate for this by increasing size. But, eventually this compensation becomes inadequate. Therefore, one can envision when a stress is put on the system so more insulin is required, diabetes could result. We are exploring what factors lead to this diminution of insulin message and the possibility that we can prevent or reverse it.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00876-02 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regenerating (REG) gene: A paracrine B-cell growth factor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Perfetti Visiting Associate LCP, NIA

Others:

J.M. Egan Senior Staff Fellow LCP, NIA  
M.E. Zenilman Special Volunteer LCP, NIA  
A.R. Shuldiner Special Volunteer LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Director of the Division of Geriatric Medicine & Gerontology,  
Johns Hopkins University School of Medicine  
L. Adams, Lab Technician, Johns Hopkins University

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Treatment of rats with poly ADP-ribose inhibitors such as nicotinamide, after 90% pancreatectomy results in pancreatic regeneration and amelioration of diabetes. Terazono and coworkers have shown that the regenerating pancreas greatly overexpresses a gene designated REG, which encodes for a novel 165 amino acid protein. In addition, recent studies have shown that REG expression may parallel islet physiology. REG gene mRNA has been shown to be undetectable when endogenous insulin synthesis is suppressed, and to be restored when endogenous insulin synthesis is normalized.

We hypothesize that REG may be a crucial autocrine and/or paracrine growth factor during embryogenesis as well as for maintenance of B-cell function in the adult. Therefore, we believe that alterations of the REG gene expression may be involved in the progressive B-cell dysfunction with aging and diabetes.

We plan to use molecular techniques to measure mRNA levels of REG in the pancreas of rodents during the normal aging process, and in rodent strains with genetic forms of type I diabetes (NOD mouse, BB rat), and type II diabetes (db/db mouse, fa/fa rat).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00877-02 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular mechanisms of insulin secretion and incretin action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Montrose-Rafizadeh Staff Fellow LCP, NIA

Others:

J. M. Egan	Senior Staff Fellow	LCP, NIA
Y. Wang	Visiting Fellow	LCP, NIA
O. Nativ	Visiting Fellow	LCP, NIA
M. Raygada	Visiting Fellow	LCP, NIA

COOPERATING UNITS (if any)

Jesse Roth, Director, Division of Geriatric Medicine and Gerontology  
Johns Hopkins University School of Medicine  
L. Adams, Lab Technician, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIH, NIA, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Non-insulin dependent diabetes mellitus (NIDDM) is a common problem in the elderly population of the USA and in the rest of the world. The aims of this project are: to find regulatory signals controlling insulin secretion from beta cells of the pancreas; to study the mechanism of action of gastrointestinal hormones called incretins (e.g. glucagon-like peptides [GLP] and glucose insulinotropic peptide [GIP]) on stimulation of insulin secretion after short term exposure; and to study the mechanism of action of incretins on beta cells proliferation after long-term exposure.

Incretins have many desirable effects in addition to stimulation of insulin secretion. They inhibit glucagon secretion from pancreatic alpha cells and enhance insulin action at insulin target tissues. The cloned rat GLP receptor has been transfected into different cell lines to study further the signal transduction involved in GLP action. Furthermore, the screening of peptides with structural similarity to GLP will allow the understanding of structure-function relationship and the development of new therapeutic agents for NIDDM.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00878-02 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1993

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Screening for mutations in target genes causing type II diabetes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. S. Celi Visiting Fellow LCP, NIA

Others:

A. R. Shuldiner Special Volunteer LCP, NIA

J. Roth Special Volunteer LCP, NIA

COOPERATING UNITS (if any)

L. Adams, Johns Hopkins University School of Medicine, Division of Geriatric Medicine & Gerontology

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Mutations in the insulin receptor gene are known to be a cause of some rare forms of extreme insulin resistance. The relevance of mutations in the insulin receptor gene as well as other candidate genes in the pathogenesis of the more common aging-related type II diabetes mellitus are currently unknown. Some evidence indicates that one rare form of diabetes mellitus, MODY, is associated with mutations in the glucokinase gene. Likewise mutations in the insulin receptor gene; insulin gene; and in mitochondrial DNA have been associated with rare forms of the disease. Current methods used to screen for mutations in candidate genes (SSCP-DGGE-ASO) are PCR-based and would miss large gene deletions (as well as gene amplifications). For this purpose we have developed a novel PCR-based method, gene dose-PCR (gd-PCR), which allows rapid and sensitive screening for gene deletions as well as gene amplifications (Celi et al. Nucleic Acid Res. 1993;21(4):1047, Celi et al. Exp.Clin.Endocrinol. 1993;101(Suppl2):330-332). gd-PCR is extremely versatile and has been successfully used for the prenatal diagnosis of trisomy 21 (Down's syndrome) from amniocytes. We have also screened the tyrosine kinase domain (exons 17,18,19,20 and 21) as well as exons 3,9 and 14 of the insulin receptor gene in five type II diabetic Mexican Americans finding no deletions. We plan to continue screening for deletions/amplifications with gd-PCR as well as screening with conventional methods such as SSCP for point mutations in the insulin receptor gene of these subjects. We also plan to study other candidate genes such as IRS-1 (insulin receptor substrate-1), glucokinase, the other hexokinases, glycogen synthase and the glucose transporters.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00879-01 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In search of insulin-receptor specific protein tyrosine phosphatase(s)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. K. Kole Senior Staff Fellow LCP, NIA

Others:

A. S. Liotta Special Expert LCP, NIA  
M. Bernier Senior Staff Fellow LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Division of Geriatric Medicine & Gerontology  
Johns Hopkins University School of Medicine  
L. Adams, Lab Technician, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIH, NIA, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Insulin resistance in obesity and aging is an expression of a common form of non-insulin dependent diabetes mellitus. We have been studying the regulation of insulin receptor phosphorylation in an *in vitro* system in an attempt to understand how this regulation can be manipulated *in vivo* as an approach to the treatment for elderly diabetics.

We have demonstrated rapid phosphorylation and dephosphorylation of the insulin receptor in solubilized membranes. Dephosphorylation of the insulin receptor by membrane-bound protein tyrosine phosphatases was specifically and substantially reduced by the presence of several new inhibitory peptides. The same peptides were ineffective in preventing a range of other (non-specific broad spectrum) phosphatases from dephosphorylating insulin receptors. The high specificity of action of several of these peptides was shown; they were effective with insulin receptor but not with the closely related EGF receptor. With intact cells, results were consistent with observations made with the permeabilized cell system.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201AG00880-01 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Arginine and nitric oxide effects on insulin release and glycogen synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.M. Egan Senior Staff Fellow LCP, NIA

Others:

C. Montrose Staff Fellow LCP, NIA  
M. Bernier Senior Staff Fellow LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine  
L. Adams, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Non-insulin dependent diabetes mellitus (NIDDM) is associated with deranged insulin secretion. When an increased insulin output is required due to peripheral resistance to insulin, the  $\delta$ -cells of the pancreas no longer respond adequately. We are studying factors that lead to defective secretion and to the increased peripheral resistance. It had been shown that the constitutive nitric oxide synthase is present in beta cells of the pancreas. Therefore, a possibility arose that nitric oxide might be required for adequate glucose-mediated insulin release. However, using a competitive inhibitor to nitric oxide synthase in the presence of various glucose concentrations, we failed to detect an effect on insulin release. In the process of evaluating nitric oxide as a potential second messenger in fat cells, we failed to detect nitric oxide synthase. However, we did find that amino acids, specifically arginine and glutamate, increased glycogen synthesis in response to insulin. We plan to investigate this further and ascertain if this modulation of glycogen synthesis by amino acids might be affected in NIDDM.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201AG00881-01 LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glucagon-like peptide (GLP-1) enhances insulin-mediated glucose uptake in adipocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.M. Egan Senior Staff Fellow LCP, NIA

Others:

C. Montrose	Staff Fellow	LCP, NIA
Y. Wang	Visiting Fellow	LCP, NIA
M. Bernier	Senior Staff Fellow	LCP, NIA
T. Henderson	Science Lab Tech	LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Division of Geriatric Medicine and Gerontology,  
Johns Hopkins University School of Medicine  
L. Adams, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

GLP increases glucose-mediated insulin release, and hence is termed insulinotropic. But, the possibility that it might have effects in the periphery arose from GLP infusions in humans. We found GLP receptors to be present in numerous cell types besides islets of Langerhans. We looked at the effects of GLP on insulin-mediated glucose uptake and lipid synthesis in 3T3-L1 adipocytes and found that GLP has stimulatory effects. We plan to investigate its role in other tissues, such as liver and muscle.



## Applied Physiology Section -- Summary

Work in the Applied Physiology Section has focused primarily on two diseases of the bone and joints, osteoarthritis (OA) and osteoporosis.

As part of the ongoing studies of OA in the Baltimore Longitudinal Study of Aging (BLSA) we have published data on radiographic hand OA in men and women, and have evaluated the association of metabolic and physiologic factors with the presence and distribution of hand and knee OA in both sexes, and with the progression of hand OA in women. In women, hand OA progresses more rapidly with increasing age. After age adjustment, body composition and bone mass are not independent predictors of hand OA. The prevalence of knee OA increases with age in both sexes, almost 50% of subjects aged  $\geq 70$  have definite knee OA, and bilateral disease accounts for close to 50% of cases in both sexes aged  $\geq 60$ . After adjustment for age, obesity was significantly associated with the presence of knee OA in both sexes, while bone density was not. We find a significant association in both sexes between hand and knee OA, supporting the concept of polyarticular OA.

Age-related changes in bone mass have been demonstrated in both men and women. Age and sex related differences in hormones involved in bone turnover are important in elucidating changes in bone physiology in normal aging and disease. The relationship of growth hormone (GH) as estimated by serum insulin-like growth factor-1 (IGF-1) to measures of obesity, fat distribution, muscle mass, and bone mineral density, was evaluated in 222 men and 131 women ranging from 20 to 94 years of age. These normal volunteers from the Baltimore Longitudinal Study of Aging had highly significant negative correlations of age with IGF-1. At the same time IGF-1 was negatively correlated with estimates of obesity, fat distribution and bone mineral density, and positively correlated with muscle mass. However, these variables are also related to age, and when the effect of age was analyzed by multiple regression techniques there was no age-independent contribution of IGF-1 to any of these parameters.

Future studies will emphasize the changes in body composition and bone in the cohort of peri-menopausal women newly recruited to the BLSA as they traverse the menopause. Studies in these women will be augmented by more frequent visits, timed to a specific stage of their cycle. This increased frequency of measurement beyond the 2 year cycle of the BLSA is necessary given the rapid changes occurring with the menopause.



## Applied Physiology Section -- Summary

Work in the Applied Physiology Section has focused primarily on two diseases of the bone and joints, osteoarthritis (OA) and osteoporosis.

As part of the ongoing studies of OA in the Baltimore Longitudinal Study of Aging (BLSA) we have published data on radiographic hand OA in men and women, and have evaluated the association of metabolic and physiologic factors with the presence and distribution of hand and knee OA in both sexes, and with the progression of hand OA in women. In women, hand OA progresses more rapidly with increasing age. After age adjustment, body composition and bone mass are not independent predictors of hand OA. The prevalence of knee OA increases with age in both sexes, almost 50% of subjects aged  $\geq 70$  have definite knee OA, and bilateral disease accounts for close to 50% of cases in both sexes aged  $\geq 60$ . After adjustment for age, obesity was significantly associated with the presence of knee OA in both sexes, while bone density was not. We find a significant association in both sexes between hand and knee OA, supporting the concept of polyarticular OA.

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Future studies will emphasize the changes in body composition and bone in the cohort of peri-menopausal women newly recruited to the BLSA as they traverse the menopause. Studies in these women will be augmented by more frequent visits, timed to a specific stage of their cycle. This increased frequency of measurement beyond the 2 year cycle of the BLSA is necessary given the rapid changes occurring with the menopause.





PROJECT NUMBER  
Z01 AG 00022-17 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone Loss with Age: Epidemiological, Familial and Cross-Cultural Considerations.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

C.C. Plato Ph.D.	Sr. Research Geneticist	LCP NIA
J.D. Tobin M.D.	Chief, Applied Physiology	LCP NIA

COOPERATING UNITS (if any)

University Zagreb, Croatia; Nihon University, Tokyo; University of Maryland; Francis Scott Key Medical Ctr.; Johns Hopkins University; CNS, NINDS.

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

2.20

0.90

1.30

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the fourth decade of life, the human skeleton begins to lose bone. That is, bone mass decreases in relation to bone volume. Menopause and the associated estrogen deficiency will enhance bone loss in females. It has also been suspected that bone loss is familial, mainly because of the increased prevalence of osteoporosis in relatives, although there are no satisfactory scientific data to support either a familial or a genetic control of bone loss. In long bones, cortical bone is resorbed from the endosteal surface. Because of the thinning of the cortical bone shell, bones lose their mechanical integrity and fracture more readily. The trabecular bone mass of the vertebral column also decreases with age. Vertebral compression fractures and fractures of the femoral neck are the most serious consequences of bone loss. This project deals with the epidemiological, genetic, cross-sectional, longitudinal, and biomechanical aspects of bone loss (1) among the participants of the Baltimore Longitudinal Study of Aging (BLSA), (2) in genetic isolates of the Adriatic Sea Islands of Croatia and the island of Guam in Micronesia and other parts of the world (cross-cultural), (3) senior athlete population, (4) in rats and dogs.



PROJECT NUMBER  
Z01 AG 00021-30 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Normal Human Variability and Cross-cultural Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

C.C. Plato	Sr. Research Geneticist	LCP NIA
J.D. Tobin	Chief Applied Physiology	LCP NIA

COOPERATING UNITS (if any)

CNS NINDS; CPSP NCI; University of Maryland; Indiana University;  
University of Zagreb, Croatia.

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology  
SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

0.30

0.20

0.10

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input checked="" type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project represents an ongoing collaborative effort, involving WHO and other national and international laboratories to coordinate the collection, evaluation and interpretation of normal genetic markers in order to study the cross-cultural patterns of genetic and extraneous factors as they relate to normative aging and to diseases with late onset, including bone loss, Alzheimer's disease, breast cancer, amyotrophic lateral sclerosis, and Parkinsonism dementia. Specifically, the objectives of this study are: A) To study the cross cultural patterns of genetic and non-genetic factors in an effort to better understand the process of normative aging. B) To study the genetic segregation of these markers in families with late onset diseases, such as Alzheimer's disease, breast cancer, ALS and others, in an effort to establish genetic linkages and eventual identification of the factors responsible for these diseases. C) To study the distribution of dermatoglyphics, lateral dominance and other genetic variables in BLSA participants and other control samples, as well as in patients with late onset diseases.



PROJECT NUMBER  
Z01 AG 00028-17 LCP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiological and Genetic Studies of ALS Complex of Guam

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

C.C. Plato	Sr. Research Geneticist	LCP NIA
J.D. Tobin	Chief Applied Physiology	LCP NIA

OTHER INVESTIGATORS:

J. Bailey-Wilson	Louisiana State University
R.C. Elston	Louisiana State University

COOPERATING UNITS (if any)

R.M. Garruto, Supervisory Research Biologist CNS NINDS  
D.C. Gajdusek, Chief, CNS NINDS

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0

CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input checked="" type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Amyotrophic Lateral Sclerosis (ALS) and Parkinsonism dementia (PD) are neurological degenerative disorders that occur in three high incidence foci in the western Pacific: among the Chamorro of Guam and the northern Mariana islands, among the Japanese on the Kii peninsula and among the Auyu and Jakai peoples of West New Guinea. Eventhough, clinically ALS and PD as seen on Guam are two distinct disorders. preliminary analyses indicated that combining all three diagnoses (ALS, PD, and ALS+PD) into one affected diagnosis for genetic analyses, was reasonable. An Age, sex and birth cohort-specific liability was defined and segregation analysis was performed under both logistic and normal models for this liability at the time of disease onset. Under either model, purely environmental, Mendelian dominant and Mendelian recessive hypotheses could be rejected, but a two allele additive major locus hypothesis could not be rejected.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 AG 00290-08 LCP

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Osteoarthritis and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

J.D. Tobin	Chief, Applied Physiology Section	LCP NIA
C.C. Plato	Senior Research Geneticist	LCP NIA
Others:F. Wigley	Chief, Rheumatology	John Hopkins Sch.of Medicine
W. Scott	Radiologist	Johns Hopkins Hospital
M. Hochberg	Rheumatologist	U. Maryland Medical School
M Lethbridge-Cejku	Research Associate	U. Maryland Medical School

COOPERATING UNITS (if any)

Johns Hopkins Sch of Med (Wigley); Johns Hopkins Hospital (Scott),  
University of Maryland Med Sch (Lethbridge-Cejku and Hochberg)

LAB/BRANCH

Laboratory of Clinical Physiology  
SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, Maryland 21224

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

0.4

0.4

0

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Osteoarthritis (OA) is the most common rheumatic disease of the elderly. As part of the ongoing studies of OA in the Baltimore Longitudinal Study of Aging (BLSA) we have published data on radiographic hand OA in men and women, and have evaluated the association of metabolic and physiologic factors with the presence and distribution of hand and knee OA in both sexes, and with the progression of hand OA in women. In 182 women with longitudinal hand xrays, hand OA progresses more rapidly with increasing age. After age adjustment, body composition and bone mass are not independent predictors of hand OA. We developed a reliable atlas of knee OA for use in both clinical and epidemiologic studies. Using this atlas, we found that the prevalence of knee OA in 547 men and 351 women increases with age in both sexes, almost 50% of subjects aged  $\geq 70$  have definite knee OA, and bilateral disease accounts for close to 50% of cases in both sexes aged  $\geq 60$ . In 465 men and 275 women aged  $\geq 40$  years, after adjustment for age, obesity was significantly associated with the presence of knee OA in both sexes, while bone density was not. Serum levels of Insulin-like growth factor-1 and urinary excretion of pyridinoline cross-links were inversely related to knee OA grade in 158 men and 100 women aged 19-86 years, but were no longer associated with OA after adjustment for age. In 431 men and 273 women aged  $\geq 40$  years, after adjustment for age, we find a significant association in both sexes between hand and knee OA, supporting the concept of polyarticular OA.





## CLINICAL IMMUNOLOGY SECTION, LCP, ANNUAL REPORT 1993

### SECTION SUMMARY

The research in the Section is directed to the exploration of cellular activation and the effects of an activation process. The findings have shown that the age related defect in T lymphocyte function is related to lost or inappropriate activation pathways. Those pathways associated with the activation of the T cell antigen receptor and the synthesis of IL-2 show an age related decline in activity. However there are pathways either directly activating the PKC dependent chain of events or the CD2 non TCR related pathway which do not demonstrate an age related decline in activity. Furthermore there is an increase in the degree of apoptosis seen when cells from old mice or old humans are activated as compared to the results seen using cells from the young. To explain this finding we have demonstrated a series of enzymes responsible for signal transduction through their ability to phosphorylate proteins as well as determining the state of gene expression for several of the important genes that are associated with the apoptotic process. The changes seen in the response of the cells from the old to an activation process include increases in the synthesis of various lymphokines by these cells to levels far above that seen for cells from young donors. To understand this and to determine what effect this might have on cellular function in the old host we have instituted a project to detail the pattern of lymphokine synthesis both in vivo and in vitro by the T cells from old and young healthy and ill individuals. Also, we are investigating the expression of the genes for the lymphokines that are made at greater levels by the cells from old donors to determine the control and promotion of these genes in the cells. This investigation involves the study of DNA binding proteins and their role in gene expression. The work in the section will continue in these directions.

The HIV project is concerned with the immune response to the HIV associated antigens. In an HIV infection there is a response not only to HIV viral antigens but also tissue antigens which seem to be either bound to the HIV peptides or have an antigenic homology with the HIV antigens. The results of an "anti-self" immune reaction in an HIV infected person may further accelerate loss of T cells and increase tissue destruction. It is also possible that the combination of the HIV and self antigens forms a superantigen which leads to a restriction of a T cell response to the virus.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00096-20-LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte Activation and Function in Aging Individuals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. A. Brock Research Biologist LCP, NIA

Other: W. H. Adler Medical Officer, PHS LPS, NIA

F. J. Chrest Biologist LPS, NIA

COOPERATING UNITS (if any)

Dr. H. J. Hoffman, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Receptor mediated activation of many cell types is followed by motility related events. In T lymphocytes, lateral redistribution of surface receptors is accompanied by aggregation of actin and myosin in cytoplasmic subcaps and both are impaired in T cells from older individuals. Age-related changes both in basal levels of filamentous actin and in further polymerization of actin after activation of resting T cells from C57BL/6 mice with Concanavalin A were previously documented. Because of technical problems using flow cytometry to document receptor mediated actin polymerization in populations of CD4 plus CD8 positive cells, a new procedure was developed to isolate CD4 and CD8 T cells immunomagnetically. Resting lymphocytes, isolated with discontinuous Percoll gradients, were incubated with magnetic beads coated with specific monoclonal antibodies to mouse T lymphocyte surface antigens. Coated beads were less efficient than T-cell recovery columns in removing B lymphocytes. Resting T cells were then incubated with CD4 or CD8 antibodies or antibody-coated beads. CD4 or CD8 positive cells, negatively selected in a magnetic field, were 98-100 percent viable, and contamination by the eliminated cell eliminated types was less than 1.0 percent. The cells were functional and responded to stimulation with the polymerization of actin to levels observed previously in unseparated resting T cell populations.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00104-17-LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.)

Clinical Immune Survey of Longitudinal Project Participants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. H. Adler Medical Officer, PHS LCP, NIA  
Other: J. E. Nagel Medical Officer, PHS LCP, NIA  
L. Song Visiting Associate LCP, NIA  
M. M. Schoonmaker, Biologist, LCP, NIA; F. J. Chrest, Biologist, LCP, NIA;  
G. D. Collins, Biologist, LCP, NIA; R. S. Pyle, Biologist, LCP, NIA;  
B. A. Dorsey-Cooper, Biologist, LCP, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.6

PROFESSIONAL:

1.6

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These studies use participants in the Baltimore Longitudinal Study of Aging and human cell lines to gain insight into the biochemical and molecular mechanisms underlying age-associated changes in immune function. Recent data indicates that human T lymphocytes activated through different cellular pathways display distinct patterns of protein phosphorylation, cytokine synthesis and gene expression, only some of which are age affected.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00441-06-LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Host Factors Relating to HIV Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. H. Adler	Medical Officer, PHS	LCP, NIA
Other:	J. E. Nagel	Medical Officer, PHS	LCP, NIA
	G. D. Collins	Biologist	LCP, NIA
	M. Schoonmaker	Biologist	LCP, NIA
	R. S. Pyle	Biologist	LCP, NIA
	B. A. Dorsey-Cooper	Biologist	LCP, NIA

COOPERATING UNITS (If any)

Dr. J. Bartlett, Dept. Medicine, Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.6

PROFESSIONAL:

.8

OTHER:

1.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immune response to the HIV involves an "autoimmune" component in which the patient makes both anti-HIV antibody as well as anti-HLA antibody. This type of response can also be seen in other viral infections which can cause immune deficiencies. During an HIV infection it appears that the older patients suffer a more extensive loss of T helper cells and reach lower levels more rapidly than do younger patients. Younger HIV infected patients can actually go through extended periods in which their CD4+ cell level remains in the normal range.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00095-20-LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Cell Membrane Structures on Cellular Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. H. Adler Medical Officer, PHS LCP, NIA

Other: J. E. Nagel Medical Officer, PHS LCP, NIA

F. J. Chrest Biologist LCP, NIA

S. M. Papciak Staff Fellow LCP, NIA

COOPERATING UNITS (if any)

Drs. R. Winchurch and D. Kittur, Department of Surgery, FSKMC, Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.05

PROFESSIONAL:

1.35

OTHER:

.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There are many cell free factors which are associated with an inflammatory response which are able to be induced in older animals and which are expressed at higher levels than seen in younger mice. The biologic implications of this finding are very important and demonstrate that the immune deficiency of aging is not a simple loss of function or cells but a change in the control of cellular function. The expression of receptors for the factors as well as an analysis of their ability to generate cellular signals in the cells from young and old mice will provide evidence for the biologic role of these factors in the older individuals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00093-21-LCP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Basis or Regulation of the Humoral Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. A. Nordin	Research Chemist	LCP, NIA
Other:	T. K. Kwon	Visiting Fellow	LCP, NIA, EOD 10/92
	M. A. Buchholz	Biologist	LCP, NIA
	F. J. Chrest	Biologist	LCP, NIA

COOPERATING UNITS (If any)

Dr. J. Shaper and N. Shaper, Oncology Center, The Johns Hopkins University  
School of Medicine, Baltimore, MD 21205

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.2

PROFESSIONAL:

2

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

A significant fraction of resting T-cells derived from old mice when polyclonally activated failed to complete the G1 phase of the cell cycle and underwent apoptotic cell death. The transcription of several genes known to be critical during the G1 phase was shown to be significantly reduced in the activated T-cells of the old mice. *In situ* hybridization data showed a reduction in the number of positive cells as well as reduced level of specific mRNA being produced in the responsive cells from the old mice. Activated T-cells blocked late in G1 expressed normal levels of specific mRNA for transferrin receptors, IL-2 and IL-2R  $\alpha$  and  $\beta$  as well as the corresponding proteins. The blocked cells were however unable to maintain normal levels of specific mRNA for c-myc and cdk2 and there was no detectable cdc2 mRNA. The presence of c-myc antisense during the activation process also inhibited the expression of cdc2 and cdk2 and the activated T-cells failed to traverse G1/S indicating a role for c-myc in the G1 that involves the transcriptional regulation of the cyclin dependent kinases.



## **ENDOCRINOLOGY SECTION:**

Important questions regarding menopausal hormone replacement therapy (HRT) include (a) what are the optimal route, dose, agents, schedule and duration of treatment, and target populations to achieve the optimal risk/benefit ratio and (b) what are the mechanisms of action of HRT at bone and other tissues? We compared the effects of estrogen replacement by oral vs. transdermal routes and found: (1) an age-independent decrease in GH secretion attributable to android body habitus; (2) a more android body fat distribution with increasing age (3) oral estrogen increased GH secretion probably due to an decrease in serum IGF-I levels; (5) transdermal estrogens decreased mean GH peak amplitude, with no change in 12 h mean GH secretion or in serum IGF-I. In another study on the effects of combination estrogen/progestin therapy on bone, lipids, and clinical symptoms in women >65 years old we have found: (1) significant decreases in total cholesterol and LDL cholesterol and increases in HDL cholesterol; (2) no changes in triglycerides, BMI, or blood pressures; (3) non-significant decreases in urinary and serum calcium, consistent with a small decrease in bone resorption. Many normal old people have reduced serum levels of IGF-I and GH secretion compared to those in young persons. GH is anabolic, increasing muscle and bone mass and reducing body fat in GH deficient young and old patients. We are investigating the relationship of GH deficiency to aging and testing the effectiveness of alternatives to recombinant human GH treatment. We have found that 6 weeks of GHRH treatment produces: (1) significant increases in GH secretion and in mean serum IGF-I levels; (2) significant increases in muscle strength; (3) significant decreases in area under glucose tolerance curves and in LDL cholesterol; (4) no changes in triglycerides or HDL cholesterol; (5) no apparent effect on body mass index, other anthropometric measures or measures of calcium metabolism. New studies will evaluate (1) the transition from pre- to postmenopause; (2) the effects of GHRH and estradiol on bone and muscle in osteoporotic women; (3) the effects of 6 months of treatment of 160 men and women >65 years with rhGH, sex steroid, both, or neither on multiple relevant clinical and physiologic end-points; (4) the effects of an oral GH secretagogue on GH and IGF-I secretion and on clinical endpoints in frail elderly women. In correlative laboratory studies, we are examining the effects of administration of GH, testosterone, or both in young and old intact male mice on gene activation in various tissues. Initial results show (1) increased total mRNA in tissues from GH treated animals, (2) an increase in EF-1 $\alpha$  stable RNA by GH or T treatment but with no additive effect. Continuation of this work will employ RNA'se protection assay of IGF-I mRNA and quantitative PCR determinations of IGF-I receptor and tissue specific protein mRNA's. Future work should expand to include estimation of IGF-I and IGF-I receptor protein by Western blotting and nuclear run-off assays to establish whether the increases observed in EF-1 $\alpha$  represent regulation at the transcriptional level.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormones, Hormone Receptors, and Aging. IV. Hormone Replacement in Menopausal Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

S. Mitchell Harman, M.D., Ph.D., Acting Section Chief, ES, LCP, NIA

Michele F. Bellantoni, M.D., Guest Scientist, ES, LCP, NIA

Marc R. Blackman, M.D. Guest Scientist, ES, LCP, NIA

Janet Vittone, M.D., Guest Scientist, ES, LCP, NIA

Robin Roberson, B.S., Chemist, ES, LCP, NIA

Shannon Hazzard, Summer Student, ES, LCP, NIA

COOPERATING UNITS (if any)

Dr. Jordan Tobin, Chief Applied Physiology Section, LCP, NIA

Jay Shapiro, M.D. Div. of Geriatrics, Depts. of Medicine, Francis Scott Key Medical Center and Johns Hopkins University School of Medicine.

Katie Bass, M.D., Dept of Obstetrics & Gynecology, Francis Scott Key Medical Center

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

1.6

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In a study of body composition, sex steroids, GH and IGF-I in postmenopausal women whose body fat distribution and body fat mass independently range from lower (gynoid) to upper (android) distribution and from lower (lean) to higher (obese) percent fat, we have found increased percent body fat and a more android body fat distribution with increasing age. There were independent inverse correlations of waist to hip ratio, but not of age or body mass index (BMI), with 12 hour pulsatile GH levels and GHRH-stimulated GH levels, suggesting an age-independent decrease in GH secretion attributable to android habitus. After oral estrogen treatment, there was increase in spontaneous GH secretion, but transdermal estrogens tended to decrease mean GH peak amplitude, with no change in 12 h mean GH secretion. Oral, but not transdermal estrogens significantly reduced basal and stimulated IGF-I. Another study examining estrogen-progestin co-therapy in older women has shown significant decreases in total cholesterol and LDL cholesterol with increases in HDL cholesterol, but no changes in triglycerides, BMI, or blood pressures. Non-significant decreases in urinary and serum calcium are consistent with a decrease in bone resorption. Results to date are consistent with potential beneficial effects of ERT on osteoporosis and coronary vascular disease in older women. New studies are being initiated to evaluate the transition from pre- to postmenopause and the effects of GHRH and estradiol on bone and muscle in osteoporotic women.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormones, Hormone Receptors, and Aging. III. Aging and Human Endocrine Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

S. Mitchell Harman, M.D., Ph.D., Acting Section Chief, ES, LCP, NIA

Marc R. Blackman, M.D. Guest Scientist, ES, LCP, NIA

Michele F. Bellantoni, M.D., Guest Scientist, ES, LCP, NIA

M. Janette Busby-Whitehead, M.D., IPA Fellow, ES, LCP, NIA

Thomas M. Stevens, M.D., IRTA Fellow, ES, LCP, NIA

Janet Vittone, M.D., Guest Scientist, ES, LCP, NIA Robin Roberson, B.S., Chemist, ES, LCP, NIA

COOPERATING UNITS (if any)

Reubin Andres, M.D., Chief, Metabolism Section & Branch Chief, LCP, NIA

William Adler, M.D., Chief, Immunology Section, LCP, NIA

Jordan Tobin, M.D., Chief, Human Performance Section, LCP, NIA

Richard Spencer, M.D., Ph.D., Senior Investigator, Laboratory of Molecular and Cellular Biology, NIA

Jesse Roth, M.D., Chief, Diabetes Unit, LCP, NIA & Chief Geriatrics, Johns Hopkins U. School of Medicine

Alan Shuldiner, M.D., Assoc. Prof., Division of Geriatric Medicine, Johns Hopkins U. School of Medicine

Richard Bennett, M.D., Asst. Prof., Division of Geriatric Medicine, Johns Hopkins U. School of Medicine

Kerry Stewart, Ed.D, Division of Cardiology, Johns Hopkins U. School of Medicine

Edward Shapiro, M.D., Asst. Prof., Division of Cardiology, Johns Hopkins U. School of Medicine

Ben Caballero, M.D., Chief, Center for Human Nutrition, Johns Hopkins University School of Hygiene

Ben Hurley, Ph.D., Chief, Dept. Kinesiology, University of Maryland

Marc Rogers, Ph.D., Asst. Prof., Dept. Kinesiology, University of Maryland

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD

TOTAL MAN-YEARS: PROFESSIONAL:

OTHER:

2.1

1.6

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We studied 12 healthy men >65 years old overnight for sleep and nocturnal GH secretory profiles, as well as for glucose tolerance, IGF-I, T, osteocalcin, lipid profiles, and bone biochemistries (urine pyridinoline cross-links, and calcium), body composition, muscle strength and utilization of high energy phosphate by NMR, all before and at the end of a regimen of GHRH 1-29 self-injected sc once nightly for 6 weeks. There was a significant increase in GH secretion after GHRH injections, in mean serum IGF-I levels, and in muscle strength and significant decreases in area under glucose tolerance curves and in LDL cholesterol, but no changes in triglycerides or HDL cholesterol and no apparent effect of treatment on body mass index, other anthropometric measures or measures of bone metabolism. A study of the effects of 6 months of treatment of 160 men and women >65 years with rhGH, sex steroid, both, or neither on multiple relevant clinical and physiologic end-points has been initiated and is ongoing.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormones and Aging. Hypothalamic-Pituitary Function in Experimental Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

S. M. Harman, M.D., Ph.D., Section Chief, ES, LCP, NIA

M. R. Blackman, M.D. Guest Scientist, LCP, NIA

Robin Roberson, B.S., Chemist, LCP, NIA

David Shock, B.S., Biologist, LCP, NIA

Zhong-Ding Lu Ph.D., Visiting Scientist, LCP, NIA

Kalonji Collins, Summer Student, LCP, NIA

## COOPERATING UNITS (if any)

Alan Shuldiner, M.D., Assoc. Prof., Division of Geriatric Medicine, Johns Hopkins U. School of Medicine

George S. Roth, Ph.D., Section Chief, MPGS, LCMB, NIA

Atsushi Miyamoto, Ph.D. Visiting Scientist MPGS, LCMB, NIA

## LAB/BRANCH

Laboratory of Clinical Physiology

## SECTION

Endocrinology Section

## INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, Maryland

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

0.3

OTHER:

1.8

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

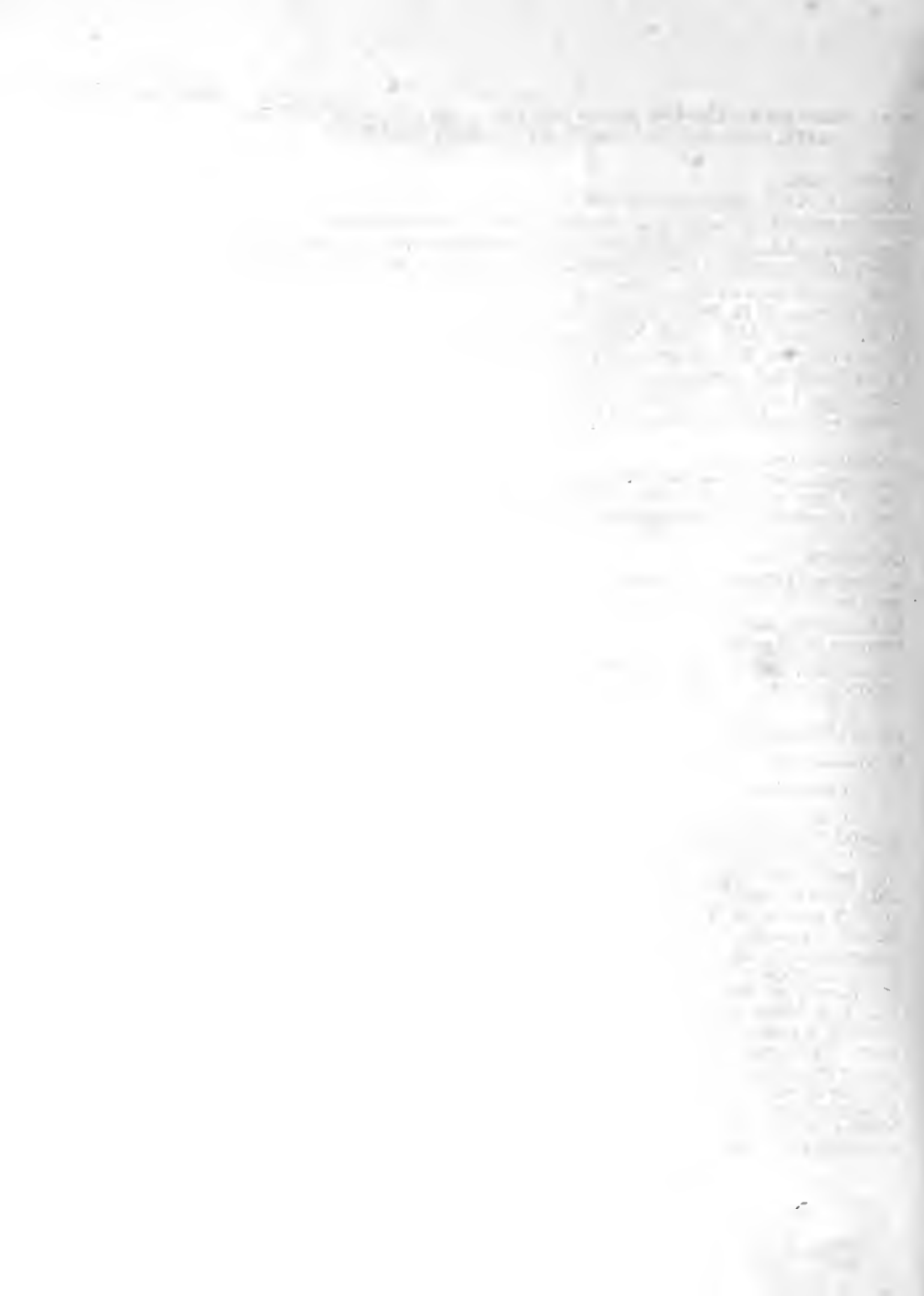
☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In an investigation of the effects of age on tissue responses to hormones, young and old male mice were treated with rhGH, T, GH plus T, or placebo and mRNA extracts were prepared from various tissues and probed by Northern blotting, RNA'ase protection assay, or template specific quantitative PCR to quantify specific mRNA's for IGF-I, IGF-I receptor, IGF-I binding proteins 1 and 3, and elongation factor 1- $\alpha$  as well as tissue specific differentiation factors and structural proteins (e.g. in muscle, myogenin and  $\alpha$ -myosin heavy chain; in bone  $\alpha$ -1 procollagen). Pretreatment, IGF-I levels were significantly lower in old mice, and plasma T and the ratio of EF-1 $\alpha$ /18S mRNA were reduced, but not significantly so. After rhGH treatment, IGF-I levels tended to increase, but this was significant only in old mice after 5 days. After 10 days, treatment with rhGH alone increased the ratio of EF-1 $\alpha$ /18S mRNA similarly in mature and old mice, whereas treatment with T alone increased EF-1 $\alpha$ /18S mRNA only in old mice. Combination treatment with rhGH + T increased EF-1 $\alpha$ /18S mRNA significantly after 5 days in mature mice. These data suggest that, in male mice, EF-1 $\alpha$  gene expression can be increased by GH or T, but with no additive response to GH + T. Aging appears to affect the pattern of this response. Continuation of this work should include estimation of IGF-I and IGF-I receptor protein by Western blotting and nuclear run-off assays to establish whether the increases observed in EF-1 $\alpha$  represent regulation at the transcriptional level.



LN

EDBP

LMG

LPC



## LABORATORY OF MOLECULAR GENETICS

### OVERVIEW:

In the Laboratory of Molecular Genetics we are studying cellular responses to stress and how they are modulated during the aging process. Some of these responses are elicited by DNA damage, and we are interested in DNA repair processes and in the induction of specific genes. We also study cellular stress reactions that do not appear to be elicited by DNA damage such as the heatshock response. We are interested in responses to exogenous damage such as ionizing irradiation, chemotherapeutics, and UV irradiation, and in responses to endogenous damage such as that caused by metabolic processes. We are increasingly interested in responses to oxidative stress. This is relevant because oxidative processes occur continuously in our cellular metabolism, and because this type of damage has been found to increase with aging. We are measuring DNA damage and repair of oxidative lesions, and we are also studying the induction of specific genes after exposure of tissue culture cells to different types of oxidation. We are then interested in whether these processes change throughout the aging process.

In the following, we describe 8 projects within LMG. Four of these are on DNA repair. We are studying the mechanism and fine structure of the repair processes with a regard to gene specific DNA repair and the preferential repair of active genes. In almost all organisms in which the gene specific repair has been studied, it appears that there is preferential repair of active genes. This is the case even in *E. coli*. A notable exception in human cells is the clinical condition, Cockayne Syndrome. Those patients are characterized by premature aging, and we are now actively pursuing whether more general forms of senescence also are associated with deficiencies in gene specific DNA repair. Another of our other projects in LMG involves the heatshock response and its regulation. We find that there is an attenuation of these responses with the aging process, and the mechanism of this change is under investigation. Two of our projects deal with the characterization of the growth arrest DNA damage inducible gene *gadd153*, the regulation of this response and the role of this protein in the stress response. Two of our projects deal with the responses to oxidative stress, the induction of specific early response genes, and the repair of the damage induced in DNA.

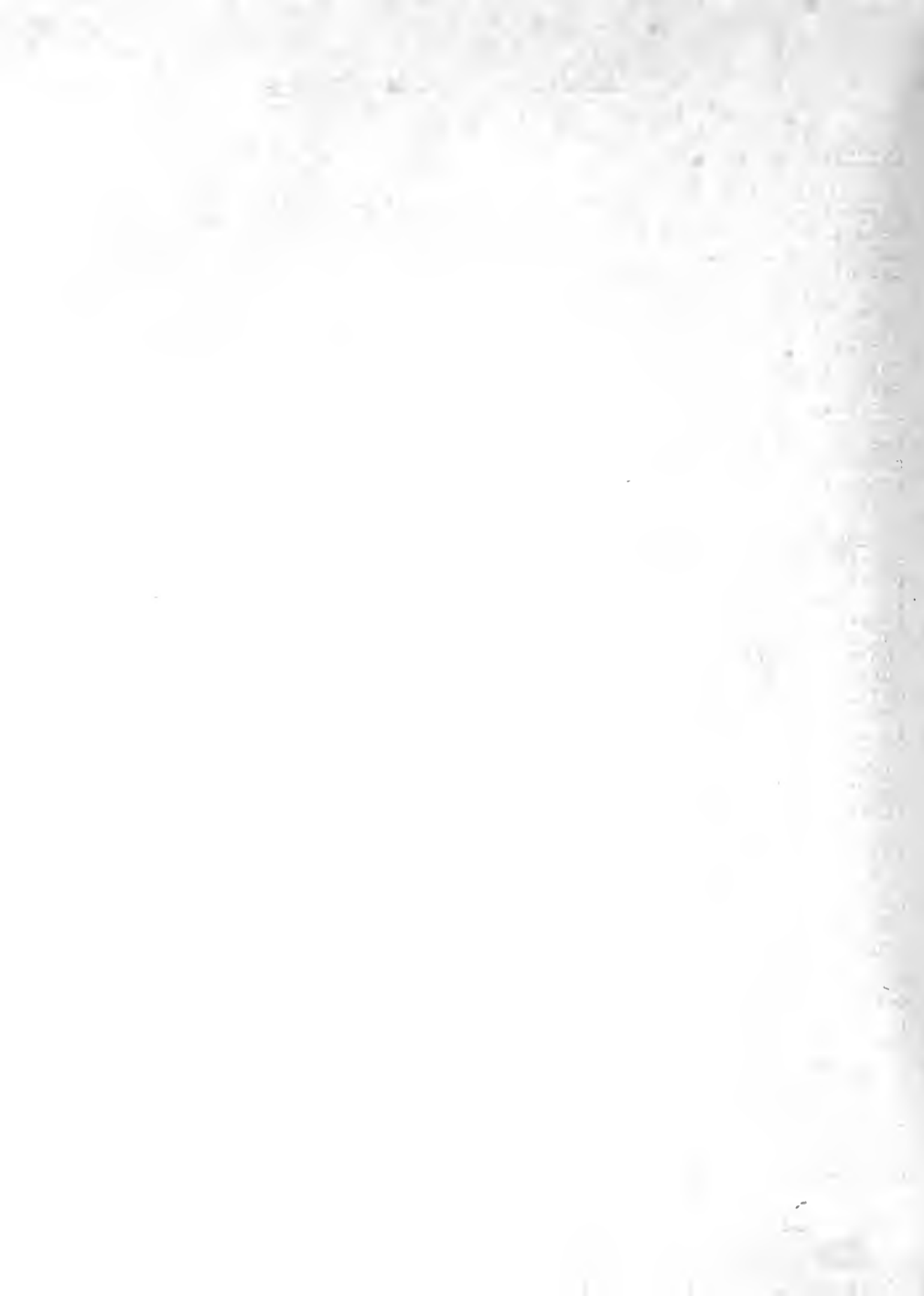
### DNA DAMAGE AND REPAIR

#### Gene Specific DNA Repair:

We are studying the molecular biochemistry of gene specific DNA repair with a view to clarify which gene products are involved and how these processes are regulated as compared to the DNA repair processes in the general, overall bulk of the genome. There are distinct differences in the efficiency of gene- and strand specific DNA repair dependent upon the type of DNA damage, and it is possible that the local degree of chromosomal distortion after DNA damage is the important element in determining the repair response chosen by the cell. We are suggesting that proficient DNA repair is necessary to secure genomic stability, and we find that certain regions of the genome that undergo translocation or rearrangements in the tumor cells are poorly repaired in cells that are susceptible to cancer.

#### Gene Specific DNA Repair Throughout the Cell Cycle:

We are interested in the regulation of DNA repair in the different phases of the mammalian cell cycle. In particular we wish to understand the regulation of preferential gene repair and of the strand bias of DNA repair





throughout the cell cycle. This is important for our understanding of the mechanism of DNA repair and for its role in mutagenesis and aging (senescent cells are thought to accumulate in G1).

We have found that in the G1 phase of the cell cycle, active genes are repaired much more efficiently than inactive regions and that the transcribed DNA strand is preferentially repaired. We are developing protocols that will allow us to characterize the DNA repair in individual genes and DNA strands in the S, and G2 phases of the cell cycle.

We have measured the cellular survival and DNA repair after UV irradiation in cells that carry a mutated p53 tumor suppressor gene. Such cells have decreased resistance and deficient DNA repair which could imply a direct role of the p53 in the repair process. Alternatively, the lack of functional p53 gene product has been shown to result in abrogation of G1 arrest in response to DNA damage. The lack of arrest may not provide the cells with sufficient time to carry out DNA repair and that could explain our result.

#### DNA Repair in Cancer and Senescence:

We are studying the role of DNA repair processes in cancer and senescence. The goal is to determine whether there are detectable deficiencies in DNA repair in these two related conditions.

Gene specific DNA repair has been measured in cells from patients with cancer prone and premature aging syndromes. Whereas we can not detect changes or deficiencies in the cancer prone syndromes, there is a lack of preferential DNA repair of active genes in at least one premature aging syndrome, Cockayne syndrome.

In the human syndrome, Fanconi's anemia, we find that there is a deficiency in DNA repair, for both DNA lesions introduced by cisplatin.

We have investigated the role of DNA repair in drug resistance. In cisplatin resistant human ovarian cancer cell lines we found a gene specific DNA repair alteration: an increase in the DNA repair efficiency of cisplatin interstrand crosslinks. This may be an important element in the resistant phenotype.

Telomeric length is one of the best available biomarkers of aging. A shortening of telomeric length with aging has been observed in many biological systems. We are measuring DNA damage and repair in these critical end-regions of the chromosomes that are also very important for genomic stability.

#### Repair of Oxidative DNA Damage:

We have been developing assays to detect oxidative lesions in specific genes and thus to quantitate their formation and repair. We generate oxidative DNA damage by several different approaches including treatment of cells with hydrogen peroxide, X-irradiation, irradiation with methylene blue, and 4NQO which generates at least one adduct with oxidative characteristics. These assays presently work very well in vitro and we are employing them increasingly in vivo to characterize the gene- and strand specific DNA repair of 8-OH guanosine and other lesions in human and hamster DNA.

While it has been a general notion that there is no DNA repair in **mitochondria**, we now find that these organelles do have repair capacity. They can not, however, repair all types of lesions. They are capable of repairing DNA lesions created by monofunctional alkylating agents, but not



of UV induced pyrimidine dimers. We have different indications that there may be efficient repair of at least some types of oxidative damage in mitochondrial DNA, and this is under investigation. We are also investigating whether the common deletions in mitochondrial DNA seen in senescence and other conditions could be due to a localized deficiency in DNA repair.

## GENE EXPRESSION AND AGING

### Heat Shock Protein Gene Expression in Response to Stress and Aging:

Heat shock proteins (HSPs), a group of highly conserved proteins induced in response to a variety of cellular stresses, appear to be critical for maintaining cellular homeostasis. Previously we demonstrated that restraint or immobilization stress elicits the induction of HSP70 mRNA and protein selectively in the adrenal gland and vasculature of rats. In both tissues this stress-induced HSP70 expression was found to be linked to the activation of the neuroendocrine response to stress and was attenuated with age. Preliminary evidence also suggested that it was mediated through the activation of one or more transcription factors known as heat shock transcription factor(HSF), which binds to a DNA sequence, the HSE, in the promoter of HSP genes resulting in their transcriptional activation. We have extended our previous studies to show that adrenal extracts obtained from aged rats show a reduced level of HSE-binding activity (indicative of less activated HSF) compared to those of young animals. Using antibodies to distinct members of the HSF family we have shown that the HSF that mediates the response is HSF1. We have compared the quantities of HSF1 protein in extracts of old and young rats and found them to be similar. These findings suggest that the differences in HSP70 expression observed between young and aged rats are not due to an age-related decline in levels of the transcription factor, but rather, are likely due to differences in a step in the signal transduction pathway leading to activation of HSF1 to a DNA binding state.

We have developed a cross-transplantation model to address the question of whether the aging process is inherent to the aorta or whether it is the environment in which the aorta resides that is responsible for the age-associated decline in stress-induced HSP70 expression in this tissue. Results obtained thus far indicate that when aortas of aged animals are transplanted to young animals, they show enhanced expression of HSP70 mRNA relative to native aortas of aged animals. In contrast, when young aortas are transplanted to aged animals, they show a marked attenuation in the response to restraint. Thus, the environment in which the aorta resides is a major factor in determining the level of HSP70 expression seen in vessels of stressed animals.

### Regulation of GADD153 by Growth Arrest, Differentiation, and Metabolic Stresses:

*GADD153* is a mammalian gene whose expression is increased in response to a variety of stresses including DNA damage and growth arrest. It is a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcriptional activators and may serve as a negative regulator of other C/EBPs. In this project, studies have focused on determining the regulation and function of *GADD153* expression in response to diverse growth inhibitory and metabolic stimuli. Studies during the past year have concentrated on the induction in response to 1) glucose deprivation, 2) treatment with the growth inhibitory prostaglandin  $PGA_2$ , and 3) during the acute phase response (APR).

1) Previously, we had shown that glucose deprivation induces *GADD153* mRNA expression in a reversible fashion. We have extended these studies to



show that the *GADD153* protein is similarly expressed and have characterized the response in detail. Induction of the gene is not due to lack of ATP as alternative energy sources (pyruvate) can not prevent the induction. C/EBPs have been implicated in the differentiation of adipocytes and *GADD153* Expression is also increased during this process. We have provided evidence, however, that *GADD153* is not required for differentiation, but rather occurs as a result of glucose depletion due to the high rate of metabolism of the differentiating cells.

2) We have provided evidence that induction of *GADD153* by  $\text{PGA}_2$  is mediated via elevations in intracellular calcium.  $\text{PGA}_2$  treatment results in an increase in intracellular calcium levels, and both this increase and the induction of *GADD153* expression by  $\text{PGA}_2$  can be blocked by buffering intracellular calcium.

3) We have found that *GADD153* is induced during the APR in rats following their injection with lipopolysaccharide. Induction of *GADD153* is temporally delayed relative to that of other C/EBPs which also show elevated expression during the APR. We have provided evidence that C/EBP beta plays a role in regulating *GADD153* expression.

#### *GADD153*, a Growth Arrest and DNA Damage Inducible Gene: Regulation by DNA Damage:

Although all cells respond to oxidative stress and DNA damage with the induction of numerous genes, little is known about the underlying mechanisms involved in these responses in eucaryotes. *GADD153* is a CCAAT/enhancer-binding protein (C/EBP)-related gene that is rapidly and highly induced in response to a variety of stresses including DNA damage and oxidative stress and provides a useful model for examining the molecular mechanisms involved in eliciting the response to genotoxic stress. It is a putative negative regulator of other C/EBP transcriptional activators, and as such could play a key role in determining what other genes are expressed or repressed in response to these stresses. Our recent studies have concentrated on the pathways mediating the induction of *GADD153* in response to DNA damage and oxidative stress. In contrast to transcriptional activation of other DNA damage inducible genes, *GADD153* induction appears to involve neither protein kinase C nor tyrosine kinases, but rather requires an unidentified serine-threonine kinase, thus representing a unique pathway in the cellular response to genotoxic stress. In addition, based on their relative sensitivity to cellular levels of glutathione, it appears that oxidative stress and DNA damage regulate transcription through different pathways. Using gel mobility shift assays we have identified several cis elements in the promoter region of the *GADD153* which show enhanced binding to factors present in nuclear lysates prepared from cells treated with DNA damaging agents. These are likely to represent binding of transcription factors important in mediating the gene's transcriptional activation in response to the stress.

To explore the relationship between DNA damage and/or repair capacity and the expression of *GADD153* we examined the activation of the *GADD153* promoter in cisplatin-resistant versus cisplatin resistant HeLa cells following their treatment with the genotoxic agent. We observed lower *GADD153* expression in the resistant cells relative to the sensitive cells from which they were derived. These findings support the notion that the expression of *GADD153* is related to the cell's sensitivity to cytotoxic effects of the DNA damaging agent.

#### Response to DNA Damage and Oxidative Stress During Cellular Aging:

Oxidative stress and DNA damage play a critical role in the development of age-associated degenerative diseases, and may underlie the aging process itself. Since cells respond to DNA damaging agents with the induction of



various genes encoding proteins which are presumed to impart protection to the damaged cell, we initiated studies to examine whether the molecular response to DNA damage is altered as a function of aging. Normal differentiated cells undergo a finite number of divisions in culture before entering an irreversible state of growth arrest. This phenomenon, termed cellular senescence, is believed to reflect certain aspects of cellular aging *in vivo*. We have compared the expression of DNA-damage inducible genes in early passage and senescent WI-38 human lung fibroblasts. We have found that with cellular-aging there is a decline in the activation of activator protein 1 (AP-1) transcription factor complexes to a DNA-binding state in response to DNA damage. This reduced AP-1 binding activity is associated with reduced expression of collagenase, whose induction following UV radiation is mediated via an AP-1 binding element. Since AP-1 complexes are comprised of fos and jun proteins, both of which have been shown to be induced in response to DNA damage and oxidative stress, we examined whether fos and jun expression following DNA damage was altered with *in vitro* cellular aging. Comparison of early and late passage WI-38 fibroblasts showed no difference in the levels of expression of either *c-fos* or *c-jun* mRNA or protein in response to DNA damage. Thus, the reduced AP-1 binding activity seen in *in vitro* aging appears to reflect changes in posttranslational events leading to the activation of AP-1 complexes to a DNA-binding state. Like collagenase, many other DNA damage-inducible genes contain an AP-1 binding site which is presumed to play a role in mediating their expression in response to genotoxic stress. Thus, an age-related loss in AP-1 transcription factor complex activation could have a major impact on the cellular response to DNA damage.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00710-05 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Heat Shock Protein Gene Expression in Response to Stress and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Nikki J. Holbrook Senior Investigator LMG, NIA

Others:

Timothy Fawcett Staff Fellow LMG, NIA

Sherrie Reichenbaugh Biologist LMG, NIA

COOPERATING UNITS (If any)

Department of Surgery, The Johns Hopkins University and Hospital (R. Udelsman)  
Laboratory of Biochem., Mol. Biol. and Cell. Biol., Northwestern University (R. Morimoto)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Heat shock proteins (HSPs), a group of highly conserved proteins induced in response to a variety of cellular stresses, appear to be critical for maintaining cellular homeostasis. Previously we demonstrated that restraint or immobilization stress elicits the induction of HSP70 mRNA and protein selectively in the adrenal gland and vasculature of rats. In both tissues this stress-induced HSP70 expression was found to be linked to the activation of the neuroendocrine response to stress and was attenuated with age. Preliminary evidence also suggested that it was mediated through the activation of one or more transcription factors known as heat shock transcription factor(HSF), which binds to a DNA sequence, the HSE, in the promoter of HSP genes resulting in their transcriptional activation. We have extended our previous studies to show that adrenal extracts obtained from aged rats show a reduced level of HSE-binding activity (indicative of less activated HSF) compared to those of young animals. Using antibodies to distinct members of the HSF family we have shown that the HSF that mediates the response is HSFl. We have compared the quantities of HSFl protein in extracts of old and young rats and found them to be similar. These findings suggest that the differences in HSP70 expression observed between young and aged rats are not due to an age-related decline in levels of the transcription factor, but rather, are likely due to differences in a step in the signal transduction pathway leading to activation of HSFl to a DNA binding state.

We have developed a cross-transplantation model to address the question of whether the aging process is inherent to the aorta or whether it is the environment in which the aorta resides that is responsible for the age-associated decline in stress-induced HSP70 expression in this tissue. Results obtained thus far indicate that when aortas of aged animals are transplanted to young animals, they show enhanced expression of HSP70 mRNA relative to native aortas of aged animals. In contrast, when young aortas are transplanted to aged animals, they show a marked attenuation in the response to restraint. Thus, the environment in which the aorta resides is a major factor in determining the level of HSP70 expression seen in vessels of stressed animals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00722-02 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of GADD153 by Growth Arrest, Differentiation, and Metabolic Stresses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Nikki J. Holbrook Senior Investigator LMG, NIA

Others:

Colette ap Rhys	IRTA Fellow	LMG, NIA
Sara G. Carlson	Biologist	LMG, NIA
Augustine M.K. Choi	Guest Researcher	LMG, NIA
Timothy Fawcett	Staff Fellow	LMG, NIA
Sherrie Reichenbaugh	Biologist	LMG, NIA
Yusen Lui	Visiting Fellow	LMG, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

2.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

GADD153 is a mammalian gene whose expression is increased in response to a variety of stresses including DNA damage and growth arrest. It is a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcriptional activators and may serve as a negative regulator of other C/EBPs. In this project, studies have focused on determining the regulation and function of GADD153 expression in response to diverse growth inhibitory and metabolic stimuli. Studies during the past year have concentrated on the induction in response to 1) glucose deprivation, 2) treatment with the growth inhibitory prostaglandin  $PGA_2$ , and 3) during the acute phase response (APR).

1) Previously, we had shown that glucose deprivation induces GADD153 mRNA expression in a reversible fashion. We have extended these studies to show that the GADD153 protein is similarly expressed and have characterized the response in detail. Induction of the gene is not due to lack of ATP as alternative energy sources (pyruvate) can not prevent the induction. C/EBPs have been implicated in the differentiation of adipocytes and GADD153 expression is also increased during this process. We have provided evidence, however, that GADD153 is not required for differentiation, but rather occurs as a result of glucose depletion due to the high rate of metabolism of the differentiating cells.

2) We have provided evidence that induction of GADD153 by  $PGA_2$  is mediated via elevations in intracellular calcium.  $PGA_2$  treatment results in an increase in intracellular calcium levels, and both this increase and the induction of GADD153 expression by  $PGA_2$  can be blocked by buffering intracellular calcium.

3) We have found that GADD153 is induced during the APR in rats following their injection with lipopolysaccharide. Induction of GADD153 is temporally delayed relative to that of other C/EBPs which also show elevated expression during the APR. We have provided evidence that C/EBP beta plays a role in regulating GADD153 expression.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00723-02 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GADD153, a Growth Arrest and DNA Damage Inducible Gene: Regulation by DNA Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Nikki J. Holbrook Senior Investigator LMG, NIA

Others:

Jennifer D. Luethy	Biologist	LMG, NIA
Augustine Choi	Guest Researcher	LMG, NIA
Helen B. Eastman	Guest Researcher	LMG, NIA
Kate Guyton	NRC Fellow	LMG, NIA

COOPERATING UNITS (If any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although all cells respond to oxidative stress and DNA damage with the induction of numerous genes, little is known about the underlying mechanisms involved in these responses in eucaryotes. GADD153 is a CCAAT/enhancer-binding protein (C/EBP)-related gene that is rapidly and highly induced in response to a variety of stresses including DNA damage and oxidative stress and provides a useful model for examining the molecular mechanisms involved in eliciting the response to genotoxic stress. It is a putative negative regulator of other C/EBP transcriptional activators, and as such could play a key role in determining what other genes are expressed or repressed in response to these stresses. Our recent studies have concentrated on the pathways mediating the induction of GADD153 in response to DNA damage and oxidative stress. In contrast to transcriptional activation of other DNA damage inducible genes, GADD153 induction appears to involve neither protein kinase C nor tyrosine kinases, but rather requires an unidentified serine-threonine kinase, thus representing a unique pathway in the cellular response to genotoxic stress. In addition, based on their relative sensitivity to cellular levels of glutathione, it appears that oxidative stress and DNA damage regulate transcription through different pathways. Using gel mobility shift assays we have identified several *cis* elements in the promoter region of the GADD153 which show enhanced binding to factors present in nuclear lysates prepared from cells treated with DNA damaging agents. These are likely to represent binding of transcription factors important in mediating the gene's transcriptional activation in response to the stress.

To explore the relationship between DNA damage and/or repair capacity and the expression of GADD153 we examined the activation of the GADD153 promoter in cisplatin-resistant versus cisplatin resistant HeLa cells following their treatment with the genotoxic agent. We observed lower GADD153 expression in the resistant cells relative to the sensitive cells from which they were derived. These findings support the notion that the expression of GADD153 is related to the cell's sensitivity to cytotoxic effects of the DNA damaging agent.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00724-01 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Specific DNA Repair

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Vilhelm A. Bohr	Chief	LMG, NIA
Others:	Alfred May	Microbiologist	LMG, NIA
	David Orren	Staff Fellow	LMG, NIA
	Florence Larminat	Visiting Fellow	LMG, NIA
	Michele Evans	Senior Investigator	LMG, NIA
	Cynthia Haggerty	Biologist	LMG, NIA
	Edward Beecham	IRTA Fellow	LMG, NIA
	Patricia Kruk	Visiting Fellow	LMG, NIA

COOPERATING UNITS (If any)

University of Texas, Smithville (R. Nairn); University of Texas at Galveston (S. Wilson); Rotterdam, The Netherlands (J. Hoeijmakers); Leiden, The Netherlands (L. Mullenders)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

5

PROFESSIONAL:

3

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying the molecular biochemistry of gene specific DNA repair with a view to clarify which gene products are involved and how these processes are regulated as compared to the DNA repair processes in the general, overall bulk of the genome. There are distinct differences in the efficiency of gene- and strand specific DNA repair dependent upon the type of DNA damage, and it is possible that the local degree of chromosomal distortion after DNA damage is the important element in determining the repair response chosen by the cell. We are suggesting that proficient DNA repair is necessary to secure genomic stability, and we find that certain regions of the genome that undergo translocation or rearrangements in the tumor cells are poorly repaired in cells that are susceptible to cancer.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00725-01 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Specific DNA Repair Throughout the Cell Cycle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Vilhelm A. Bohr Chief LMG, NIA

Others:

David Orren	Staff Fellow	LMG, NIA
Lone Petersen	Guest Researcher	LMG, NIA
Nicholas Rampino	Staff Fellow	LMG, NIA
Florence Larminat	Visiting Fellow	LMG, NIA
Bonita Taffe	Staff Fellow	LMG, NIA

COOPERATING UNITS (If any)

Columbia University (A. Carothers; D. Grunberger); Johns Hopkins University (M. Kastan); National Cancer Institute, NIH (C. Harris)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are interested in the regulation of DNA repair in the different phases of the mammalian cell cycle. In particular we wish to understand the regulation of preferential gene repair and of the strand bias of DNA repair throughout the cell cycle. This is important for our understanding of the mechanism of DNA repair and for its role in mutagenesis and aging (senescent cells are thought to accumulate in G1).

We have found that in the G1 phase of the cell cycle, active genes are repaired much more efficiently than inactive regions and that the transcribed DNA strand is preferentially repaired. We are developing protocols that will allow us to characterize the DNA repair in individual genes and DNA strands in the S, and G2 phases of the cell cycle.

We have measured the cellular survival and DNA repair after UV irradiation in cells that carry a mutated p53 tumor suppressor gene. Such cells have decreased resistance and deficient DNA repair which could imply a direct role of the p53 in the repair process. Alternatively, the lack of functional p53 gene product has been shown to result in abrogation of G1 arrest in response to DNA damage. The lack of arrest may not provide the cells with sufficient time to carry out DNA repair and that could explain our result.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AG 00726-01 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**DNA Repair in Cancer and Senescence**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Vilhelm A. Bohr Chief LMG, NIA

Others:

Michele K. Evans	Senior Investigator	LMG, NIA
Cynthia Haggerty	Biologist	LMG, NIA
David Webb	IRTA Fellow	LMG, NIA
Patricia Kruk	Visiting Fellow	LMG, NIA

COOPERATING UNITS (if any)

National Cancer Institute, NIH (K. Kraemer); National Cancer Institute, NIH (C. Harris); National Cancer Institute, NIH (J. Robbins); National Cancer Institute, NIH (I. Horak); Johns Hopkins University (L. Grossman)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

3

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying the role of DNA repair processes in cancer and senescence. The goal is to determine whether there are detectable deficiencies in DNA repair in these two related conditions.

Gene specific DNA repair has been measured in cells from patients with cancer prone and premature aging syndromes. Whereas we can not detect changes or deficiencies in the cancer prone syndromes, there is a lack of preferential DNA repair of active genes in at least one premature aging syndrome, Cockayne syndrome.

In the human syndrome, Fanconi's anemia, we find that there is a deficiency in DNA repair, for both DNA lesions introduced by cisplatin.

We have investigated the role of DNA repair in drug resistance. In cisplatin resistant human ovarian cancer cell lines we found a gene specific DNA repair alteration: an increase in the DNA repair efficiency of cisplatin interstrand crosslinks. This may be an important element in the resistant phenotype.

Telomeric length is one of the best available biomarkers of aging. A shortening of telomeric length with aging has been observed in many biological systems. We are measuring DNA damage and repair in these critical end-regions of the chromosomes that are also very important for genomic stability.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00727-01 LMG

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Repair of Oxidative DNA Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Vilhelm A. Bohr Chief LMG, NIA

Others:

Bonita Taffe	Senior Staff Fellow	LMG, NIA
Nicholas Rampino	Staff Fellow	LMG, NIA
Florence Larminat	Visiting Fellow	LMG, NIA
Edward Beecham	IRTA Fellow	LMG, NIA
Alfred May	Microbiologist	LMG, NIA

COOPERATING UNITS (if any)

Villejuif, France (F. Laval); Lab. Mol. Physiol. and Genetics, NIA (R. Cutler);  
Lab. of Biological Chemistry (C. Filburn); Munchen, Germany (S. Paabo)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been developing assays to detect oxidative lesions in specific genes and thus to quantitate their formation and repair. We generate oxidative DNA damage by several different approaches including treatment of cells with hydrogen peroxide, X-irradiation, methylene blue, and 4NQO which generates at least one adduct with oxidative characteristics. These assays presently work very well in vitro and we are employing them increasingly in vivo to characterize the gene- and strand specific DNA repair of 8-OH guanosine and other lesions in human and hamster DNA.

While it has been a general notion that there is no DNA repair in mitochondria, we now find that these organelles do have repair capacity. They can not, however, repair all types of lesions. They are capable of repairing DNA lesions created by monofunctional alkylating agents, but not of UV induced pyrimidine dimers. We have different indications that there may be efficient repair of at least some types of oxidative damage in mitochondrial DNA, and this is under investigation. We are also investigating whether the common deletions in mitochondrial DNA seen in senescence and other conditions could be due to a localized deficiency in DNA repair.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00728-01 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Response to DNA Damage and Oxidative Stress During Cellular Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Nikki J. Holbrook Senior Investigator LMG, NIA

Others: Augustine M.K. Choi Guest Researcher LMG, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

.7

PROFESSIONAL:

.7

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Oxidative stress and DNA damage play a critical role in the development of age-associated degenerative diseases, and may underlie the aging process itself. Since cells respond to DNA damaging agents with the induction of various genes encoding proteins which are presumed to impart protection to the damaged cell, we initiated studies to examine whether the molecular response to DNA damage is altered as a function of aging. Normal differentiated cells undergo a finite number of divisions in culture before entering an irreversible state of growth arrest. This phenomenon, termed cellular senescence, is believed to reflect certain aspects of cellular aging in vivo. We have compared the expression of DNA-damage inducible genes in early passage and senescent WI-38 human lung fibroblasts. We have found that with cellular-aging there is a decline in the activation of activator protein 1 (AP-1) transcription factor complexes to a DNA-binding state in response to DNA damage. This reduced AP-1 binding activity is associated with reduced expression of collagenase, whose induction following UV radiation is mediated via an AP-1 binding element. Since AP-1 complexes are comprised of fos and jun proteins, both of which have been shown to be induced in response to DNA damage and oxidative stress, we examined whether fos and jun expression following DNA damage was altered with in vitro cellular aging. Comparison of early and late passage WI-38 fibroblasts showed no difference in the levels of expression of either c-fos or c-jun mRNA or protein in response to DNA damage. Thus, the reduced AP-1 binding activity seen in in vitro aging appears to reflect changes in posttranslational events leading to the activation of AP-1 complexes to a DNA-binding state. Like collagenase, many other DNA damage-inducible genes contain an AP-1 binding site which is presumed to play a role in mediating their expression in response to genotoxic stress. Thus, an age-related loss in AP-1 transcription factor complex activation could have a major impact on the cellular response to DNA damage. Previously a part of project AG 00721-02 LMG

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10



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**ANNUAL REPORT OF THE  
LABORATORY OF PERSONALITY AND COGNITION  
NATIONAL INSTITUTE ON AGING  
1992-1993**

**Overview**

The fundamental scientific paradigm which guides research in the Laboratory of Personality and Cognition (LPC) is the analysis of individual differences. Few phenomena are more basic than the fact that human beings differ—in health, in rates of aging, in cognitive ability, in personality, in happiness and life satisfaction. The mission of the LPC is threefold: (1) to conduct basic and clinical research on individual differences in cognitive and personality processes and traits; (2) to investigate the influence of age on these variables and their reciprocal influence on health, well-being, and adaptation; and (3) to employ longitudinal, experimental, and epidemiological methods in the analysis of psychological and psychosocial issues of aging, including health and illness, predictors of intellectual competence and decline, models of adult personality, and correlates of disease risk factors.

**Basic Research on Personality Structure**

Theoretical advances in the Five-Factor Model of personality

A theoretical advance was made in the conceptualization of ego development in relation to the five-factor model (FFM) of personality structure. While both theoretical systems use the term Conscientiousness (C), it has a different meaning as applied by Loevinger in her stage model of moral cognition and by five-factor theorists who apply it to a dimension of personality relevant to moral behavior. In their commentary to a target article, LPC scientists pointed out that ego level determines the form of impulse control whereas the dimension of Conscientiousness (C) determines the degree of impulse control. In addition to making linkages between C and other dimensions of the FFM, the advantage of a dimensional system such as the FFM, is that it allows a more differentiated view than do stage models that require that individuals be assigned to one and only one category.

This same theme of categorical vs. dimensional approaches to understanding psychological phenomena is seen particularly clearly in the area of personality disorders. This year in collaboration with researchers at Johns Hopkins Behavioral



Biology Research Center and the FSK Department of Psychiatry, the FFM was used to understand anti-social personality disorder among drug abusers. Drug abusers with only anti-social personality disorders versus those with mixed or other disorders were characterized on the NEO-PI. Subsets of anti-social patients with borderline and other diagnoses were characterized by severe emotional distress and instability. A marked vulnerability to stress in mixed anti-social patients was especially noteworthy. Future applications of personality assessment information, planning and intervention treatments, and outcome studies form an important future agenda.

Other research on the FFM from the LPC and collaborators suggests that the FFM provides an adequate description of both normal and clinical populations generally. An important implication of this is the recognition that it is less fruitful to discuss normal and abnormal personality and more profitable to examine the relations between psychopathology and personality traits. In this regard, an important reformulation of psychopathology from the perspective of the FFM was provided by senior investigator McCrae, who suggests that the concept of personality disorder can be replaced by the construct of personality-related disorder defined as a set of life problems that are characteristically related to the person's personality. In addition, McCrae has described an alternative to the DSM-IV system for the diagnosis of personality-related disorders. Work on this alternative system, the development of which requires the constructing a compendium of problems in living that are associated with high or low standing on each of the five factors, is continuing.

Several papers and chapters also advanced our understanding of normal personality theory and assessment. In an invited chapter, the five-factor theory of personality is offered as a prototype for a new generation of personality theories. Various theoretical contexts for the FFM are explored, including its underlying assumptions of variability, proactivity, rationality, and scientific knowability; substantive links to individual differences identified in a variety of classical personality theories; and the FFM's place within a broad metatheoretical framework for understanding the person.

#### Empirical advances in personality assessment

The view that personality traits play a meaningful role in predicting behaviors is increasingly shared by scientists in other countries. A recent example of this is a lead article in the British journal, The Psychologist by a pair of



Scottish psychologists who advance the FFM as the most comprehensive and useful model of personality.

Conceptual analyses of other personality instruments such as the California Psychological Inventory and Lorr's Interpersonal Style Inventory suggest correspondences or links between scales from these instruments and factors from the FFM. One recent paper from LPC this year performed two studies in which judges rated the item content of CPI scales in terms of the FFM and CPI scales were correlated with factors as measured by NEO-PI. Both studies showed meaningful links between the CPI and four of FFM factors. The factor of Agreeableness, however, was under-represented in the CPI. Similarly, the second order factors of the ISI have been interpreted as measures of the FFM. However, several ISI scales showed empirical relations that were not predicted from their classification in the ISI. Such results confirm the generality of the FFM, but underscore the need for detailed empirical analyses to confirm or qualify interpretation of scales in terms of the FFM.

#### Cross-observer agreement in personality assessment

An exciting new statistical index of profile agreement and an associated coefficient (Rpa) was developed by McCrae to assess agreement across observers on personality profiles. He provided a derivation, as well as a nomogram for determining agreement between two ratings on single factor. This new agreement index and nomogram are currently employed in a study on cross-observer using married couples from the BLSA in collaboration with Dr. Peter Fagan and staff from Personality, Stress, and Coping Section.

Alternative methods of personality assessment, which complement the standardized questionnaires of the FFM, are also an important part the program of basic research on personality and personality assessment within LPC. A continuing concern in personality research is the use of self-report measures. Alternative methods of assessing personality are desirable to confirm or qualify conclusions based on self-report. Inferences about personality based on respondents' meaning structures provides a promising new method of personality assessment that can be conveniently studied using BLSA archives. To pursue this topic, the Meaning Questionnaire, which provides inferred traits in terms of 22 dimensions including the type and form of relation, was recently administered to BLSA participants.

An abbreviated version of the NEO-PI was administered to 82 pairs of identical twins and 171 pairs of fraternal twins





reared apart and to 132 pairs of identical twins and 167 pairs of fraternal twins reared together. Estimates of genetic and environmental effects for Openness and Conscientiousness were similar to those found in other studies of personality: Genetic influences were substantial and there was little evidence of shared rearing environment. Results for agreeableness were different: Genetic influence accounted for only 12% of the variance and shared rearing environment accounted for 21% of the variance. Few significant gender or age differences for genetic and environmental parameters were found.

A further test of a genetic basis of the FFM will be provided in a collaborative study with John Loehlin of the University of Texas at Austin with data from the National Merit Scholarship Qualifying Test study using items from the California Psychological Inventory (CPI) to measure the dimensions of the five-factor model.

### **Cognition and Neuropsychology**

#### Early markers of Alzheimer's disease in the BLSA.

As part of the Summer Research Fellowship Program, Ms. Karen Mae Trotman and Mr. Armando San Juan, Jr. presented a poster on age-associated changes in specific error types on the Benton Visual Retention Test (BVRT). Ms. Trotman and Mr. San Juan have completed their first year of medical school and carried out their summer research project under the supervision of Dr. Susan Resnick. While earlier studies have indicated increased BVRT errors with age, particularly after age 70, the decline in visual memory has not been examined by specific error type. Errors can be classified into 7 general categories: omissions, distortions, perseverations, rotations, mislocations, additions and size errors. In this project we examined specific error types for 2,065 present and former BLSA participants all of whom were administered the BVRT on at least one visit. Participants were divided into 7 age groups by decade, from 20-29 through age 80 and older. The initial analyses were based on cross-sectional data for administrations at the first BLSA visit, although subsequent analyses will examine longitudinal change scores. Results of the study indicated that distortions, rotations, perseverations and mislocations were the most frequent errors across all ages. Although older participants made significantly greater errors regardless of error type, the greatest age differences were found for distortions and omissions. Men and women showed similar patterns of age-associated increases in errors. In addition, there was a



significant interaction between gender and error type, indicating that women across all ages made more omissions and rotations but not other types of errors. The next steps in this project will be to examine longitudinal change in specific error types and to relate these changes to intra-individual changes in hormonal status.

In their review of the Laboratory during the past year, the Board of Scientific Counselors applauded the shift in emphasis by the Cognition Section from a focus on normal aging to research aimed at distinguishing pathological from normal cognitive aging. This change has implications for important studies on the changes underlying pathological cognitive aging, and Drs. Zonderman, Resnick, and Giambra have made excellent progress in initiating this work. The most immediate evidence of this new focus has been changes in the content of the cognitive testing program such that tests were divided into two batteries, one for longitudinal prediction and another for cognitive and neuropsychological outcomes. In addition to adding a test of visuo-spatial performance to the longitudinal predictor battery, the longitudinal study of cognition was preserved by substituting contemporary tests of verbal knowledge and of immediate and delayed memory for words and numbers for tests tapping the same cognitive domain that have been historically administered. Preliminary results have shown that these new tests are highly correlated with their predecessors, but take less time to administer and score. The cognitive and neuropsychological outcome battery was designed to tap impairment in memory, language, visuo-spatial performance, intelligence, problem solving, and attention.

#### Structural and functional brain changes

The Cognition Section has an exciting opportunity to link a neuroimaging component to the BLSA. During the past year, the Contract Advisory Committee recommended approval of our proposal to perform annual neuroimaging studies on selected BLSA participants. A contract has been written in which annual MRI and PET studies will be performed on 90 men and 90 women from the Laboratory's on-going Early Markers of Alzheimer's Disease (EMAD) study. The Board of Scientific Counselors recommended that we convene a panel of expert advisors to assist formulating these studies. We met with our expert panel in February and they reviewed and approved our proposed neuroimaging protocol.

The proposed studies will examine regional brain structures and functions using these in vivo neuroimaging techniques by performing annual measurements of regional cerebral anatomy



using MRI and regional cerebral blood flow using PET. The goals of this project are: (1) To use the wealth of prior cognitive data, particularly repeated assessments of the Benton Visual Retention Test (BVRT), to identify BLSA participants who show subsequent changes in brain structure and function consistent with cognitive changes reported by LPC investigators. For example, do prior BVRT scores predict which participants will show cognitive and brain changes? (2) To examine trajectories of cognitive change, including the full neuropsychological battery in addition to the BVRT, concurrently with brain changes. For example, do observable brain changes precede cognitive changes over the course of the 9 year study? (3) To determine the incidence, location and progression of brain abnormalities in aged subjects and their association with cognitive aging.

This project represents a unique use of the BLSA to accomplish a number of important objectives including: furthering our understanding of basic neurocognitive or brain-behavior relations; understanding normative changes in brain structure and function; and relating cognitive performance trajectories to changes in brain structure and function. This research program is a valuable addition to the LPC and will enable us to examine the neuroanatomic and neurophysiologic concomitants of normal and pathological aging. These data will be a significant complement to the neuropathology studies performed as part of the BLSA autopsy program.

The Laboratory has established an important collaboration with investigators at the Human Nutrition Research Center on Aging in which we will examine the relationship between total serum homocysteine and cognitive impairments in BLSA EMAD subjects. This work is based on previous findings in which poor performance on the Mini-Mental Status Examination was related to elevated levels of homocysteine. This project will also examine the overall influences of nutrition, including vitamin B1, B12, and folate levels, on cognitive performance using a food frequency questionnaire which measures the overall adequacy of dietary intake, as well as providing specific information about fat and fiber intake behavior.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Stress, Coping and Personality in Aging Men and WomenPRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)  
(Name, title, laboratory, and institute affiliation.)

Robert R. McCrae	Research Psychologist	LPC, NIA
Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA

COOPERATING UNITS (if any)  
Longitudinal Studies BranchLAB/BRANCH  
Laboratory of Personality and CognitionSECTION  
Personality, Stress and CopingINSTITUTE AND LOCATION  
National Institute on Aging, Gerontology Research Center,  
Baltimore, MD 21224

TOTAL MAN-YEARS: 2.1                      PROFESSIONAL: 1.1                      OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects	(b) Human tissues	(c) Neither
(a1) Minors		
(a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two studies on the longitudinal stability of personality were conducted. In the first, it was hypothesized that stability coefficients would be lower for individuals with changes in physical health. This hypothesis was not supported; instead, high stability of personality was found regardless of changes in physical health status. In the second, six-year retest data were analyzed for the California Q-Set, a configural measure of personality, which also showed evidence of stability. Longitudinal research on personality, stress and coping will continue.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Basic Research in PersonalityPRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)  
(Name, title, laboratory, and institute affiliation.)

Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Robert R. McCrae	Research Psychologist	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Personality and Cognition

SECTION

Personality, Stress and Coping

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center,  
Baltimore, MD 21224

TOTAL MAN-YEARS: 1.3

PROFESSIONAL: 1.1

OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

(b) Human tissues

(c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Personality can be defined in terms of enduring individual differences in emotional, interpersonal, experiential, and motivational styles. The five factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness provide a comprehensive taxonomy of personality traits for the description of personality in aging men and women. Two studies were conducted as part of continuing program of research on these factors. In the first, alternative measures of the five-factors were compared, and both general agreement and specific differences were found. In the second, items of the California Q-Set were used to examine the discriminant validity of facets of each of the five factors.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Psychosocial Predictors of Mental and Physical HealthPRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)  
(Name, title, laboratory, and institute affiliation.)

Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Robert R. McCrae	Research Psychologist	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA
Stephanie V. Stone	Staff Fellow	LPC, NIA
Chester A. Schmidt	Special Volunteer	FSKMC

COOPERATING UNITS (if any)  
Psychiatry Department, FSKMCLAB/BRANCH  
Laboratory of Personality and CognitionSECTION  
Personality, Stress and CopingINSTITUTE AND LOCATION  
National Institute on Aging, Gerontology Research Center,  
Baltimore, MD 21224

TOTAL MAN-YEARS: 2.8      PROFESSIONAL: 1.6      OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects	(b) Human tissues	(c) Neither
(a1) Minors		
(a2) Interviews		

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This on-going research attempts to refine the assessment of coronary prone behavior, especially the Type A behavior pattern. (TABP). Previous studies in the literature suggested that the global TABP was an independent risk factor for coronary heart disease, but more recent studies did not replicate these findings. When specific components of Type A were examined in the Multiple Risk Factor Intervention Trial (MRFIT), only hostility ratings predicted CHD ( $RR = 1.69$ ,  $p < .05$ ,  $n = 576$ ). In an attempt to improve interview-derived assessments of hostility, more reliable and valid ratings of hostility were developed based on the five factor model of personality and its conceptualization of hostility. The present research examines these new ratings as predictors of CHD morbidity and mortality.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Early Markers of Alzheimer's Disease in Longitudinal ParticipantsPRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)  
(Name, title, laboratory, and institute affiliation.)

Alan B. Zonderman	Research Psychologist	LPC, NIA
Susan Resnick	Senior Staff Fellow	LPC, NIA
Leonard M. Giambra	Research Psychologist	LPC, NIA
Claudia H. Kawas	Staff Neurologist	FSKMC
Robert R. McCrae	Research Psychologist	LPC, NIA
E. Jeffery Metter	Medical Officer	LSB, NIA
Paul T. Costa, Jr.	Chief, LPC	LPC, NIA

COOPERATING UNITS (if any)  
Longitudinal Studies Branch  
Department of Neurology, FSKMCLAB/BRANCH  
Laboratory of Personality and CognitionSECTION  
Cognition SectionINSTITUTE AND LOCATION  
National Institute on Aging, Gerontology Research Center,  
Baltimore, MD 21224

TOTAL MAN-YEARS: 5.5                      PROFESSIONAL: 2.5                      OTHER: 3.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects	(b) Human tissues	(c) Neither
(al) Minors		
(a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Participants in the BLSA aged 60 and older were examined to detect changes in psychological, neurological, and neuropsychological tests related to early signs of Alzheimer's disease (AD). Six-year changes in immediate visual memory performance assessed by the Benton Visual Retention test were used to predict AD prior to its onset and 6-15 and 16-22 year subsequent cognitive performance. Subjects with diagnoses of AD had larger changes in immediate memory performance over the six-year interval prior to the estimated onset of their disease than subjects without AD. Six-year longitudinal change in immediate visual memory performance also predicted 6-15 and 16-22 year subsequent cognitive performance, even after adjusting for the influences of age, general ability, and initial immediate memory. These results provide evidence that change in immediate visual memory performance has long-term prognostic significance over as many as 16-22 years.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)  
Attentional Processes in Normal and Impaired Elderly

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)  
(Name, title, laboratory, and institute affiliation.)

Leonard M. Giambra                      Research Psychologist                      LPC, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Personality and Cognition

SECTION

Cognition

INSTITUTE AND LOCATION National Institute on Aging, Gerontology Research Center,  
Baltimore, MD 21224

TOTAL MAN-YEARS: 1.0

PROFESSIONAL: 0.50

OTHER: 0.50

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

(b) Human tissues

(c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We seek to understand the psychological and biopsychological aspects of normal and pathological aging in terms of attention and attentional processes. We are also concerned with applying that knowledge to develop strategies for improving attentional and cognitive functioning. This year we report on an investigation into compensatory variations in sustained attention situations which result in disproportional improvements in performance in the elderly. A particularly powerful variation was the increase in exposure time of the stimuli--the elderly improved their performance while the young and middle-aged were unaffected by the increase in exposure time. We have also begun the development of an attention-switching functional test which will be used in our attempts to provide early markers of Alzheimer's Disease.





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ANNUAL REPORT OF THE LABORATORY OF NEUROSCIENCES  
NATIONAL INSTITUTE ON AGING  
1992-1993

I. ORGANIZATION AND MISSION STATEMENTS

The Laboratory of Neurosciences (LN) at the National Institute on Aging was formed in 1978, and is involved in research on the central and peripheral nervous systems in health, aging and disease, including Alzheimer disease. The Laboratory is located at the Clinical Center in Bethesda, Maryland, and is divided into three sections entitled, (1) Cerebral Physiology and Metabolism, (2) Brain Aging and Dementia, and (3) Neurochemistry and Brain Transport. In addition, there are four Units entitled (1) Positron Emission Tomography, (2) Neuropsychology, (3) Pharmacology and Pharmacokinetics, and (4) Brain Imaging and Computers. In September 1982, a six-bed temporary Patient Care Unit (PCU) was established to study inpatients with Alzheimer's disease and other dementias, as well as healthy subjects. The PCU, moved to permanent quarters on the 6D Ward in 1990. An Outpatient Clinic also was started in 1982 to screen subjects of inpatient protocols and to carry on outpatient-related research.

A. SECTION ON CEREBRAL PHYSIOLOGY AND METABOLISM (STANLEY I. RAPOPORT, CHIEF)

This section investigates the function, structure, physiology, biochemistry, and pharmacology of the central and peripheral nervous systems and the changes that take place during development and aging. Areas of investigation include the application of in vivo techniques to study brain glucose and lipid metabolism (using radioactive 2-deoxy-D-glucose or fatty acids); examination of the structure and function of the blood-brain and blood-nerve barriers, in disease models and before and following modification, in relation to chemotherapy of central nervous system disease; the use of tissue culture techniques to examine neuronal electrical properties in relation to altered genetic composition (trisomy 16 mice); the use of histological techniques to examine neuronal morphology and plasticity; neurochemical and molecular biological techniques to examine the Alzheimer brain.

B. SECTION ON BRAIN AGING AND DEMENTIA (MARK B. SCHAPIRO, CHIEF)

This section examines the metabolic, anatomical, neurochemical and neuropsychological parameters that characterize cerebral function in the following subject groups, so as to understand aging and disease of the brain and to provide a differential diagnosis of Alzheimer disease and other dementias: (1) healthy men and women at different ages; (2) dementia of the Alzheimer type; (3) Down syndrome; (4) multiple infarct dementia; (5) treated chronic hypertensives; (6) depression in the elderly. The section employs positron emission tomography to examine cerebral metabolic rates for glucose and cerebral blood flow, computerized CT and magnetic resonance imaging to evaluate brain anatomy magnetic resonance spectroscopy to evaluate brain metabolism, analytical techniques to explore the composition of cerebrospinal fluid, and neuropsychological tests to evaluate the details of cognitive function. The program has initial cross-sectional studies followed by longitudinal studies with post-mortem follow up.

C. UNIT ON POSITRON EMISSION TOMOGRAPHY (CHERYL L. GRADY, CHIEF)

This unit is responsible for developing and conducting research involving positron emission tomography on human subjects in relation to aging, dementia, including Alzheimer disease and multiple infarct dementia (see Section heading), and developmental abnormalities of the brain, including retardation. Clinical protocols are formulated to examine brain glucose utilization using 18-F-2-deoxy-D-glucose, and blood flow using 15O-water, as positron-emitting tracers. Studies are performed on subjects at rest, with reduced visual and auditory inputs, and under conditions of well-defined cognitive or physiological



stimulus paradigms. Metabolic and flow data are related to data obtained with CT and neuropsychological measures.

**D. UNIT ON BRAIN IMAGING AND COMPUTERS (BARRY HORWITZ, CHIEF)**

This unit is responsible for conducting research involving in vivo structural imaging of the human brain in healthy subjects and in the patient groups noted above. Images are obtained using magnetic resonance imaging (MRI). Quantitative volumetric analyses are performed in order to assess differences in volumes of significant brain structures (e.g., ventricles, basal ganglia), and to determine volumetric changes in individuals followed longitudinally. This Unit also conducts research on human in vivo brain phosphorus and glucose metabolism using magnetic resonance spectroscopy (MRS). In addition, this Unit conducts research involving the use of multivariate statistical methods and computer computational techniques for analyzing brain functional activity as measured by PET.

**E. UNIT ON NEUROPSYCHOLOGY (GENE E. ALEXANDER, CHIEF)**

This unit is responsible for the design, implementation and analysis of neuropsychological research on memory, language, cognition, and attention in healthy subjects and in patient groups noted above. The goal of this research is to identify and describe changes in mental abilities that are a function of age or age-related disease, to propose and test cognitive models for these changes, and to relate them to neuroanatomical, neurochemical, and physiological changes that are concurrently measured. The unit participates in clinical protocols to evaluate cognitive and behavioral effects of centrally acting drugs, including possible therapeutic agents for the treatment of Alzheimer disease.

**F. UNIT ON PHARMACOLOGY AND PHARMACOKINETICS (TIMOTHY T. SONCRANT, CHIEF)**

This unit is responsible for conducting research on the sites and modes of action of centrally acting drugs in humans, in relation to peripheral pharmacokinetics, behavioral and cognitive responses and metabolic changes within the brain. Humans are studied in relation to age and neurodegenerative disorders, including Alzheimer disease and depression. Drugs are evaluated for therapeutic efficacy, using cognitive and other measures. Cerebrospinal fluid concentrations of neurotransmitters and their metabolites are measured by analytical techniques, often developed by the unit. The unit also trains clinicians in the proper conduct of research in clinical pharmacology and therapeutics.

**G. SECTION ON NEUROCHEMISTRY AND BRAIN TRANSPORT (QUENTIN R. SMITH, CHIEF)**

The function of this section is to conduct research on the transport, distribution, metabolism, and physiological actions of critical solutes and drugs within the central and peripheral nervous systems in relation to brain function, aging and dementia. The program examines the cerebral uptake, distribution and actions of environmental toxins and metals which may have a role in brain aging and dementia. In addition, the program explores the mechanisms that regulate cerebral metabolism, protect the brain from circulating toxins, and maintain a stable ionic environment for neuronal function. The program also examines the molecular basis of transport at the blood-brain barrier, and designs drugs for enhance brain uptake in treating central nervous system disease.



## II. RESEARCH HIGHLIGHTS

This section summarizes selected research accomplishments from the Office of the Chief (Stanley I. Rapoport) and Section on Cerebral Physiology and Metabolism, not summarized under later Section or Unit Headings.

### A. SECTION ON CEREBRAL PHYSIOLOGY AND METABOLISM

#### I. MECHANISMS FOR ALZHEIMER DISEASE

1. Phylogenetic hypothesis for Alzheimer disease. Positron emission tomography demonstrates selective metabolic involvement of the frontal, parietal and temporal association neocortices in Alzheimer patients, and relative lack of involvement of primary and sensory motor regions. Furthermore, Alzheimer neurofibrillary tangles are selective to the association as compared to primary sensory and motor cortical regions, and Alzheimer neuropathology is found in nonneocortical brain regions which underwent rapid changes during recent hominid and higher primate evolution. These observations suggest that Alzheimer disease is a phylogenetic disease which involves a telencephalic system of brain regions which expanded differentially during evolution of higher primates. Some genetic changes that promoted this expansion are postulated to have made this system vulnerable to Alzheimer-type degeneration. This work was done by S. Rapoport.

2. Region-specific membrane instability in Alzheimer disease. Maintenance of cell membrane lipids within a stable bimolecular layer depends on membrane composition and body temperature. Membrane lipids from the brain spontaneously assemble into a unilamellar state in vitro, at a critical temperature  $T^*$  of 37°C.  $T^*$  is reduced by 10°C in affected but not unaffected regions of the brain in Alzheimer disease. Thus, cell membranes in these affected regions are physically unstable, and would tend to become disrupted over time. Their rate of disruption may influence the course of Alzheimer disease. This work was done by L. Ginsberg, N. Gershfeld and S. I. Rapoport.

3. Reversible synaptic failure underlies early functional deficits in Alzheimer disease. A hypothesis was put forward that early functional and metabolic deficits in Alzheimer disease reflect reversible failure of synaptic transmission. This hypothesis was based on evidence from our laboratory that brain association areas which demonstrate reduced resting glucose metabolism and blood flow can nevertheless be fully activated during an appropriate cognitive task; and on evidence that synapses are lost in excess of neurons early in Alzheimer disease. Reversible synaptic failure suggests that drug therapy be directed to prevent further loss of synaptic circuitry, and to optimize the circuitry which remains intact in early Alzheimer disease. This work was done by S. Rapoport and C. Grady.

4. New single-subject method to analyze brain blood flow responses to experimentally controlled levels of stimulation. Based on the hypothesis (section 3) that stimulation by tasks with graded levels of intensity and difficulty, using brain imaging of regional cerebral blood flow (rCBF), would provide information about mechanisms of neurotransmission failure in Alzheimer disease, a new analytic method was developed to relate rCBF, obtained with positron emission tomography (PET) or dynamic magnetic resonance imaging in individual subjects, to task difficulty and intensity. This method was applied successfully to relate PET-derived rCBF to task frequency in subjects performing a motor task. This work was done by J. Maisog, T. Zeffiro and S. Rapoport.

#### II. MOLECULAR BIOLOGY OF BRAIN AGING AND DISEASE

1. Cytochrome oxidase distribution in the primate brain. Cytochrome oxidase activity was identified by cytohistochemistry in terminal fields of long projection neurons in the monkey brain, within the perforant pathway of the hippocampal formation and entorhinal cortex, and within the neocortex. On the other hand, cytochrome oxidase mRNA was found within neurons leading to these long projections, using in situ hybridization. These results indicate that long





projection neurons, which are particularly vulnerable to Alzheimer degeneration in humans, have the enzymatic machinery and synthetic capacity for high levels of oxidative metabolism. As such, they may be the sites of mitochondrial abnormalities or of accumulation of free radicals. This work was done by K. Chandrasekaran and D. Brady.

2. Cytochrome oxidase gene expression in the Alzheimer brain. Examination of postmortem Alzheimer brain using classical staining techniques and in situ hybridization for mRNA for cytochrome oxidase II, demonstrated more than 75% losses in cells of the perforant pathway of the hippocampal formation and entorhinal cortex, parts of the brain association system that is selectively vulnerable to Alzheimer disease and that has evolved dramatically in humans (see I.1, above). These results suggest that Alzheimer type degeneration is related to high levels of oxidative metabolism in certain synaptic areas, and perhaps to mitochondrial abnormalities, synaptic dysfunction or free radical accumulation. This work was done by K. Chandrasekaran and D. Brady.

3. Parvalbumin in the Alzheimer brain. Cells containing this calcium buffering protein were reduced significantly in lateral (by 50%) and basal (by 20%) nuclei of the amygdaloid complex of the Alzheimer brain. Such cells also were reduced by 50% in the dentate gyrus and CA1 subfields of the hippocampal formation. The affected regions are part of a brain association system which is vulnerable to disease, as compared to other regions within the amygdala and hippocampal formation. These anatomic studies support the hypothesis that Alzheimer disease selectively involves neurons which compose a recently evolved brain association system (see I.1, above). This work was done by Dr. D. Brady.

4. Amyloid precursor protein (APP) synthesis and distribution. APP, thought to be pathogenic in Alzheimer disease, was shown to be selectively induced by nerve growth factor in differentiating PC12 and PC12S cells, and to localize in growth cones of neurites of PC12S but not PC12 cells. The form induced was APP695. Addition of agents which perturb calcium, decreased APP and synaptophysin levels. These results indicate a selective interaction between nerve growth factor, calcium and APP695 expression. This work was done by R. Fukuyama.

5. APP gene expression is calcium dependent. In isolated Jurkat-T cells and human peripheral T cells, APP gene expression was activated by a calcium ionophore and phorbol ester, confirming that calcium is a regulator of expression. This work was done by R. Fukuyama.

6. Hippocampal transplants from fetal trisomy 16 mouse, a model for trisomy 21 (Down syndrome). Hippocampal neurons from embryonic day 15-17 trisomy 16 and control mouse fetuses were transplanted into brains of 6-8 week old adult mice, and allowed to survive for 6-14 months. No evident morphological, immunological or molecular difference was noted between control and trisomic transplants, each of which was robust even at 14 months. Thus, despite having genes which correspond to those on human chromosome 21, including the gene for APP, excess expression of the 16th chromosome in the mouse does not lead to Alzheimer type degeneration in neural tissue. The results are consistent with the hypothesis that full blown degeneration needs the human genome in its entirety, and that Alzheimer disease is phylogenetic (See I.2 above). This work was done by J. Stoll.

7. Novel technique to identify neuron specific gene expression. An extract of human entorhinal cortex, known to be vulnerable to Alzheimer disease, was used to stimulate rat splenocytes *in vitro*. Spleens were obtained from rats that were exposed to a human brain region not known to be vulnerable, and immune suppressed by cyclophosphamide. Antibodies were generated which stained axonal/synaptic elements and neurofibrillary tangles in the temporal lobe of the Alzheimer brain, as well as elements of the limbic system in the rat brain. This selective immunization technique may help to identify why the human entorhinal cortex and not other regions develop Alzheimer pathology. This work was done by R. Fukuyama and D. Brady.



### III. REGULATION OF NEURONAL DEVELOPMENT

1. Electrophysiological properties of hippocampal neurons from the trisomy 16 mouse, a model for Down syndrome in humans (trisomy 21). Use of patch clamp showed that trisomy 16 fetal hippocampal neurons in culture had a reduced rate of depolarization of the action potential as compared to control hippocampal neurons, and a reduced voltage-dependent inward sodium current. Those differences are in the opposite direction from those observed in dorsal root ganglion human trisomy 21 and mouse trisomy 16 neurons, and suggest neuron-specific rather than a general difference in active neuronal properties in the trisomic state. This work was done By Z. Galdzicki and E. Coan.

2. Calcium currents in cultured trisomic hippocampal neurons. Whole cell inward calcium currents, recorded by patch clamp from the somatic region of cultured mouse hippocampal control and trisomy 16 neurons, were identified as high and low voltage-activated currents. The high but not the low voltage-activated current was significantly larger in the trisomic neurons, consistent with prolongation of the action potential, and indicating another difference in electrical properties which might be related to retardation in Down syndrome. This work was done by Z. Galdzicki and E. Coan.

3. N-methyl-D-aspartate (NMDA) currents in the trisomy 16 hippocampus. No difference in the voltage-independent NMDA currents was found between cultured hippocampal neurons from fetal trisomy 16 and control mice. As voltage-activated currents do differ between the populations (III-2 and 3), the defect in active electrical properties is specific and voltage-dependent. This work was done by E. Coan.

### IV. BRAIN LIPID METABOLISM, RELATION TO FUNCTION AND DISEASE

1. Mathematical model for incorporation of plasma fatty acids into brain phospholipids. A model was developed to quantitate incorporation of intravenously injected radiolabeled fatty acids into brain phospholipids in vivo. The model includes entry of the fatty acid from plasma into brain, oxidation of the fatty acid, *de novo* synthesis, and recycling from acyl-containing brain lipids. Unidirectional incorporation coefficients, calculated by an operational equation, can be used to estimate turnover of particular fatty acids in individual phospholipids. The model provides a basis for using the fatty acid method in humans, with positron emission tomography. This work was done by P. Robinson and S. I. Rapoport.

2. Incorporation of radiolabeled fatty acids into an experimental brain tumor. Rats with brain implants of Walker 256 carcinosarcoma cells were injected with radiolabeled palmitate, docosahexaenoate or arachidonate, and incorporation into and turnover within tumor and brain phospholipids, of each fatty acids, were measured using the fatty acid model of Robinson and Rapoport (IV-1, above). Each tracer labeled the tumor as compared with control brain by 4-7 fold. Labeling corresponded to increased turnovers of fatty acid within brain phospholipids. Incorporation into tumor was in the order: palmitate > arachidonate > docosahexaenoate. These results suggest that the fatty acid method can be used to image human brain tumors, and to characterize the metabolism of such tumors. This work was done by T. Nariai, J. DeGeorge and N. Greig.

3. Radiosynthesis of [1-C-11]fatty acids. In order to examine fatty acid incorporation into the human brain, using positron emission tomography, [1-C-11]positron emitting isotopes of arachidonate, palmitate and docosahexaenoate were synthesized and purified to 95%, using a synthetic step to synthesize precursors of critical Grignard reagents. This work was done by M. A. Channing.

4. Brain uptake of a radiolabeled fatty acid, as measured with positron emission tomography in the primate. The positron emitting fatty acid, [1-C-11]arachidonate, was injected intravenously in anesthetized rhesus monkeys and shown to produce quantifiable images of the brain, from which incorporation coefficients comparable (2-fold smaller) than found in the rat could be



calculated by the fatty acid method. Tracer uptake was shown to independent of cerebral blood flow, by doing studies during inhalation of carbon dioxide. The results indicate for the first time that positron emitting fatty acids can be used to examine brain phospholipid metabolism in higher primates and possibly in humans, with positron emission tomography. This work was done by M. Chang and T. Arai

5. Fatty acid incorporation to screen for neurotoxic effects of drugs. In collaboration with the Food and Drug Administration, the fatty acid method was used to examine neurotoxicity of domoic acid, which has caused memory loss or death in a number of Canadians who ate tainted mussels. Administration of domoic acid to rats demonstrated selective abnormalities in incorporation of [1-<sup>14</sup>C]arachidonate, related to glial response. This work was done by N. Appel.

6. Effect of an inhibitor of beta-oxidation on incorporation of radiolabeled palmitic acid into brain. In order to employ radiolabeled palmitic acid in humans using positron emission tomography, an attempt was made to inhibit oxidation of the tracer once it enters brain. This was shown to be feasible. In rat experiments, the drug, 2-tetradecylglycidate, an inhibitor of carnitine-acyltransferase II, the enzyme which transfers acyl-CoA from cell cytoplasm to mitochondria, was shown to enter the brain and markedly inhibit oxidation of [1-<sup>14</sup>C]palmitate. Most of the tracer was consequently found within brain phospholipids. This work was done by M. Chang.

7. Effect of acute unilateral enucleation of the eye in the rat on uptake of fatty acids into phospholipids of brain visual structures. Removal of one eye in the rat resulted, after 1 day, in reduced incorporation of the polyunsaturated [1-<sup>14</sup>C]arachidonate and [1-<sup>14</sup>C]docosahexaenoate but not of the saturated [9,10-<sup>3</sup>H]palmitate into central visual structures. The effect on the polyunsaturated fatty acids likely reflects acutely reduced functional activity coupled with reduced phospholipase A2 activation. As reduced incorporation of labeled palmitate occurs after several weeks, the results show that the fatty acid method can be used to distinguish changes in brain functional activity as compared to changes in neuroplasticity. This work was done by S. Wakabayashi.

## B. SECTION ON BRAIN AGING AND DEMENTIA

### I. GENETICS OF ALZHEIMER DISEASE (AD)

1. Transmissibility of Alzheimer disease. Alzheimer disease (AD) recently was reported to be a transmissible disease, based on evidence that a spongiform encephalopathy similar to Creutzfeldt-Jacob Disease was transmitted to hamsters by intracerebral inoculation with buffy coat from blood of patients with AD and their unaffected first-degree relatives. To examine this possibility, blood was obtained from 21 unaffected first degree relatives (families also had another affected AD member), 10 demented patients with clinically diagnosed AD and 21 healthy controls without a history of familial neurologic disease.

No hamster in either the first or second passage developed neurologic signs consistent with a spongiform encephalopathy. Blinded neuropathologic examination of sections of all first passage and most second passage hamster brains revealed no evidence of a spongiform encephalopathy. This work suggests that Alzheimer disease is not due to a transmissible agent. This work was done by M. Schapiro and M. Godec.

2. DNA Repair. Phytohemagglutinin stimulated blood lymphocytes from Down syndrome subjects showed increased chromatid aberrations, and DNA strand breaks and gaps in response to G2-phase-irradiation compared to Alzheimer disease and normal cells. An abnormal response to post-irradiation addition of cytosine arabinoside, a DNA repair inhibitor, also was demonstrated in Down syndrome cells, indicating a deficiency in incision at sites of DNA base damage. Thus, Down syndrome, like other cancer-prone genetic disorders, has a G2 DNA repair deficiency not associated with neurodegeneration. Further, DS subjects have a reduced capacity for DNA incision to remove X-ray damaged sites in DNA. Such



deficiency could result in the accumulation of unrepaired DNA damage and ultimately lead to cell death, and may be associated with a neurologic degeneration in both DS and AD. This work was done by M. Schapiro and K. Sanford.

3. Family pedigree study. We have shown that monozygotic twins concordant for AD have many more affected first degree relatives, with a lesser predicted distribution of the proportion of surviving first degree relatives without disease, than do monozygotic twins discordant for AD. This suggested that heritable and sporadic (not evidently heritable) forms of AD exist. On this basis, first degree relatives of randomly-enrolled AD probands were examined with regard to the prevalence of AD-illness, and of other disorders compared with first degree relatives of healthy controls. Among the 434 first degree relatives, older than 39 years, of the 39 AD probands, 65 secondary cases (15%) of AD-like illness were found, compared to 24 cases among 489 relatives (5%) from controls. Lifetime risk of AD was 55% by the age of 94 years for the relatives of the AD probands, as compared with 24% by 90 years among relatives of controls (factor of 2). No significant difference in lifetime risk was found between relatives, parents or siblings of probands with early compared to late onset disease, or with regard to gender. Similar prevalences of Parkinson's disease, stroke, Down syndrome, schizophrenia, hematologic malignancy, depression substance abuse and diabetes were found between relatives from controls as compared with AD probands. These studies show a 2-3 fold greater life-time risk for AD in relatives of probands with AD, indicative of heritability, but no difference in regard to onset age and cognitive decline. This work was done by Z. Wu, C. Kinslow and M. Schapiro.

## II. DOWN SYNDROME AND OTHER GENETIC DISORDERS INVOLVING THE BRAIN

1. Role of X chromosome and sex steroids in human brain development. Turner syndrome (TS) is characterized by a loss of part of one X chromosome, absence of endogenous estrogen due to ovarian involution, and retardation. In order to examine the role of estrogens and the X chromosome and brain integrity, 18 TS adults and 19 age/sex matched controls were studied with PET, magnetic resonance imaging and neuropsychological tests. Compared to controls, the TS subjects had lower memory and visuo-spatial cognitive test scores; small volumes of the hippocampus, subcortical nuclei and parieto-occipito brain bilaterally, and a reduced metabolic rate for glucose in the right parietal cortex. Within mosaic TS subjects, visuospatial scores and right parietal metabolism were correlated with percent lymphocytes having a complete 45,X karyotype. These results indicate that the X chromosome is involved in a dose dependent manner in maintaining right parietal lobe function, and that sex steroids are necessary for normal development of the hippocampus. This work was done by D. Murphy and M. Schapiro.

2. Distinctive pattern of neuropsychological impairment in old Down syndrome subjects. Down syndrome subjects develop Alzheimer type neuropathology after the age of 35 years. They concurrently become demented, and when they do they show metabolic reductions on PET in the same areas as do DAT patients, mainly in the association neocortex, as well as accelerated ventricular dilatation on quantitative computer assisted tomography. Based on these observations, it was hypothesized that cognitive decline in older DS subjects occurs in two stages that can be separated by many years. Stage 1 involves loss of some skills and coincides with accumulation of senile neuritic plaques in brain, whereas the second stage leading to full blown dementia occurs together with formation of neurofibrillary tangles and accelerated cell loss. Such a temporal sequence may also occur in premorbidly normal individuals with DAT, and can be distinguished by combining PET and volumetric imaging of the brain. This work was done by M. Schapiro.

3. Cognition and brain metabolism in fragile X [ $\text{fra(X)}$ ]. Fragile X is a genetic disorder characterized by retardation. Cognitive testing of healthy male adults with  $\text{fra(X)}$ , compared with male Down syndrome subjects with a comparable level of mental retardation, demonstrated poorer visual short term memory and





better verbal skills. Global glucose utilization did not differ between the two groups. However, metabolic asymmetry in the parietal lobe was greater in the fra(X) group, and principle component and discriminant analyses demonstrated relative metabolic elevation in the lenticular nucleus and thalamus, with altered functional relations between these regions and other parts of the brain. Thus, another genetic abnormality provides a distinctive cognitive and metabolic profile, arguing for further studies on the role of genetics (and particularly of the X chromosome) on human brain development. This work was done by M. Schapiro and D. Murphy.

#### 4. Abnormal metabolic pattern involving language areas in Down syndrome.

A discriminant function of regional glucose metabolic patterns, obtain with PET, was derived and could successfully classify young nondemented Down syndrome adults (100%) from matched healthy controls (88%). This function demonstrated abnormal metabolic interactions between brain regions known to be involved with language. despite no difference in absolute metabolic rates between DS and control subjects. Thus, there is a distinct abnormality in brain regions involved with language in Down syndrome, reflecting increased expression of genes on chromosome 21. This work was done by N. Azari, B. Horwitz and M. Schapiro.

#### 5. Neuropsychological evaluation of adults with Down syndrome.

Down syndrome subjects over 35 years of age, without clinical dementia, demonstrated significant neuropsychological abnormalities relative to young Down adults. Abilities to form new long-term memories and visuospatial construction were consistently diminished, whereas immediate memory span and language were not. These deficits may be the early consequences of Alzheimer neuropathology. Longitudinal study of older adults with Down syndrome showed that onset of dementia is associated with accelerated decline of neuropsychological function, increased rate of cerebral atrophy, and the appearance of significant reductions of association cortex metabolism. These changes in brain function and anatomy suggest that dementia onset represents more than an arbitrary point in a continuous process of degeneration. Rather, dementia onset appears to signal a fundamental change in the AD process. A mental status examination for adults with Down syndrome was developed that provides good discrimination between adults with and without clinically significant dementia. This work was done by J.V. Haxby and M.B. Schapiro.

### III. CARDIOVASCULAR DISEASE AND THE BRAIN

#### A. HYPERTENSION

1. Brain atrophy in cognitively-normal subjects with long-term controlled hypertension. Systemic hypertension affects 1 of every 4 adults in the United States, increases in prevalence with age, and is a major risk factor for vascular dementia and stroke. Seventeen neurologically normal men (mean age 68 yr) with controlled hypertension of at least 10 years duration were compared to 17 age and sex matched healthy controls by means of volumetric magnetic resonance imaging. The hypertensives had a significantly smaller left hemisphere brain volume, and 2 fold increments in the volumes of the right and left lateral ventricles, as compared with the controls. When hypertensive subjects with severe periventricular hyperintensities were removed from the analysis, the differences remained statistically significant. All hypertensives and controls were cognitively normal. These findings indicate that long-standing treated hypertension results in loss of brain mass even in the absence of clinical or measurable cognitive symptoms.

2. Cerebral metabolic patterns in mildly hypertensive patients. Resting state glucose metabolism was measured using PET in 17 mildly demented hypertensive men without cognitive deficits or non-brain end organ disease, compared with 25 age-matched control men. Data were analyzed with regard to territories of the major cerebral arteries. No significant difference in global metabolism was found between patients and controls, whereas 13 of 65 regions of interest show significant declines of approximate 10% in regional metabolism ( $p < 0.05$ ). These comparatively minimal findings contrasted with evidence,



demonstrated by a correlation analysis of PET data, of a marked loss of metabolic coupling between brain areas supplied by the anterior and middle cerebral arteries, but not by the posterior or basilar arteries. As most of the loss occurred in the watershed area between the anterior and middle cerebral circulation, it was postulated that accumulated transient hypotensive episodes in hypertensive subjects were their likely cause. The relevance of this mechanism to appearance of vascular dementia in hypertensives remains to be determined. This work was done by M. Mentis, J. Salerno and B. Horwitz.

## B. VASCULAR DEMENTIA.

Vascular Dementia. MRI scans in some demented subject show altered density in white matter termed by Hachinski leukoaraiosis, or leukoencephalopathy. This abnormality is thought to represent vascular pathology, which contributes to dementia in 40% of the demented elderly. Dementia of the Alzheimer type (DAT) patients (14) who had severe leukoencephalopathy (DAT+) were compared with those (13) who did not (DAT-) and with 14 healthy controls. Nine of the 14 DAT+ patients were hypertensive. Both DAT groups had reduced glucose metabolism, but DAT+ group had relatively more reductions in subcortical nuclei. Postmortem examination of 3 DAT+ patients demonstrated severe cerebral amyloid angiopathy of penetrating cortical arteries, suggesting that such angiopathy can be contribute to vascular abnormalities in AD. This work was done by C. DeCarli

## IV. DISEASES OF THE BASAL GANGLIA AND THEIR CONNECTIONS

1. Alzheimer-type reductions in parietal/temporal glucose metabolism can occur in Parkinson's disease. A 68 year old man with slowly progressive dementia was diagnosed as having Alzheimer disease during life, but was found to have Parkinson's disease at autopsy. Reductions in relative metabolism as measured by positron emission tomography in parietal and temporal areas were shown, similar in regional distribution and magnitude to those seen in patients with probable Alzheimer disease. These results suggest that reductions of glucose metabolism in association neocortex in Alzheimer's disease are not specific to the disease process, but maybe related to the dementia state and connections of the cortex with the basal ganglia. This work was done by M. Schapiro.

2. Familial inverted chorea. Five patients with familial inverted chorea (FIC), a progressive heritable motor disorder disease without dementia, did not demonstrate atrophy of the striatum or cortex on quantitative CT, as compared to age matched controls. However, a discriminant analysis of PET derived regional metabolic rates demonstrated abnormal functional interactions involving the caudate nucleus and putamen, regions also abnormal in Huntington's and Parkinson's disease. These study demonstrate the importance of using PET data to looking at abnormal functional interactions within brain region regulating motor movement. This work was done by P. Pietrini.

3. PET predicts drug responsiveness in obsessive compulsive disorder (OCD). Ten of 18 OCD patients who had been initial PET scans for glucose metabolism were rescanned after at least 1 year of therapy with postsynaptic serotonergic antagonist drugs (clomipramine or fluoxetine). Five of the patients showed improvement on drug and 5 did not. Both the improved and nonresponsive patients had reduced orbitofrontal metabolism on the second scan compared to the first. Among the clomipramine-treated patients, orbitofrontal glucose use was higher on the second scan of the responders than nonresponders, and was directly correlated with clomipramine plasma levels. These data indicate a role in OCD for a region in frontal cortex connected with the basal ganglia. They show for the first time for any disease that PET can be used to predict responsiveness to a therapeutic agent. This work was done by P. Pietrini, C. Grady, M. Schapiro and S. Swedo.

4. Multiple regression/discriminant analysis to assess drug responsiveness in obsessive compulsive disorder (OCD). A regression/discriminant analysis procedure, applied to PET derived glucose metabolic data from 10 patients with



OCD, as compared with controls, demonstrated that an abnormal pattern involving the basal ganglia, thalamus and limbic brain regions could distinguish patients from controls. After therapy with a serotonergic drug, clomipramine or fluoxetine (-2), all responders were classified as controls whereas those that did not respond remained in the OCD category. This results demonstrate how PET data can be used to assess drug response in disease, and argue that the serotonergic drugs actually normalized brain interactions in OCD. This work was performed by N. Azari, K. Pettigrew, S. Swedo and B. Horwitz.

## C. UNIT ON POSITRON EMISSION TOMOGRAPHY

### I. AGING

1. Cognitive activation of regional cerebral blood flow (rCBF) in aging. Object vision (face perception) and spatial vision (localization) were examined in young and old subjects in two experiments. Both young and old subjects showed occipitotemporal rCBF activation during face matching and occipitoparietal activation during location matching when these conditions were compared to the control task. However, in both experiments and in both tasks, young subjects showed greater activation of prestriate cortex (Brodmann's area 18), and old subjects had larger rCBF increases in occipitotemporal cortex (area 37). Foci in prefrontal cortex, as well as in inferior and medial parietal cortex, were more activated in the old subjects during location matching. The results of these experiments show that reliable age-related changes during visual processing can be found in rCBF patterns, suggesting more efficient use of occipital visual areas by younger subjects and more reliance by older subjects on one or more cortical networks, particularly for spatial vision, perhaps to compensate for reduced processing efficiency of occipital cortex. This work was done by C. Grady, J. Haxby, and B. Horwitz.

2. Age-related changes in rCBF and rCMRglc during a global sensory activation. Subjects were studied under two experimental conditions- at rest and during sensory activation (watching a documentary film). Significant increases of absolute and normalized rCBF and rCMRglc values were found during sensory activation bilaterally in the occipital regions in both age groups. At rest, frontal rCBF and rCMRglc were higher in the young than in the old; sensory activation induced frontal increases of rCMRglc in the young and decreases in the old, whereas no group differences in rCBF activation were seen in frontal cortex. Coupling between rCBF and rCMRglc was present in both age groups and was stronger during sensory activation. These results show that brain activation enhances age-related differences in brain metabolism, and suggest that old subjects may show more habituation over long activation periods, accounting for reduced rCMRglc, but not rCBF, during the film. This work was done by B. Horwitz and P. Pietrini.

### II. ALZHEIMER DISEASE

1. Activation of rCBF in patients with dementia of the Alzheimer type DAT. Mildly-moderately dementia DAT patients showed rCBF activation in occipitotemporal cortex in the same location as did the controls. In addition, the magnitude of the activation was not reduced compared to control levels (17% vs. 15% normalized increase). This was in spite of the fact that some patients had reduced flow in this region of cortex. The DAT patients also had increased rCBF during face matching in frontal cortex, an area not activated in controls. These results indicate that extrastriate cortex retains the ability to locally increase CBF during task performance despite reductions in flow due to disease, and that additional areas of frontal cortex are needed, possibly due to an increased demand on attention in the patients. This was done by C. Grady, J. Haxby, B. Horwitz, and M. Schapiro.

2. Glucose metabolism and quantitative neuropathology in Alzheimer's disease. Neurofibrillary tangles showed a striking predilection for association cortical regions, whereas plaques and neuronal densities had a more even distribution in brains of Alzheimer's disease patients. In addition, the tangle



densities were positively correlated with regional metabolic measures in 5 of the 6 patients, whereas plaque density and neuronal counts were not related to metabolism. This study suggests that regional metabolic abnormalities seen during life are related to the pathological processes responsible for neurofibrillary tangle formation. This work was done by C. DeCarli, C. Grady, and M. Schapiro.

3. Analysis of rCMRglc in subjects "at risk" for probable Alzheimer Disease. Two PET examinations one year apart were performed on a 65 y.o. man with isolated memory impairment and family history for autosomal dominant AD. Sixteen sex- and age-matched healthy volunteers were used for comparison. Initial rCMRglc data did not reveal any consistent abnormality as compared to controls, but a follow-up evaluation did reveal rCMRglc reductions in parietal regions, coincident with worsening of cognitive impairment. Using a discriminant function that successfully distinguished mild/moderate DAT patients from controls, the subject was correctly identified as an AD patient with 100% probability on both PET scans. These results emphasize the importance of alternative methods of analysis to increase usefulness of resting PET data in the early detection of AD. This work was carried out by P. Pietrini and N. Azari.

4. Visuospatial attention in DAT. Reaction time was measured in DAT patients and controls during a letter discrimination task with both valid and invalid cues as to target location. There were no differences between controls and patients with valid cues, but invalid cues caused greater slowing of reaction time in the patients. In addition, performance was correlated with asymmetry of metabolism in the superior parietal lobe in the patients, but not in the controls. These results indicate that focusing of attention to spatial location is intact in early DAT, but disengagement of attention is impaired. This attentional dysfunction may be linked to disruption of cortical networks linking the parietal and frontal lobes. This work was carried out by C. Grady, J. Haxby, and P. Nestor.

5. Neural mechanisms of delusions of misidentification (DMS) in DAT. Seven patients with DAT and DMS were compared with 17 DAT patients without DMS. On a wide battery of neuropsychiatric tests, compared to normals, both the DMS and DAT groups showed typical DAT abnormalities. Compared to the DAT group the DMS patients had significant hypometabolism in orbito-frontal regions bilaterally, basal ganglia and left posterior cingulate, but relative sparing in anterior and medial temporal lobes bilaterally, and parieto-temporal areas mainly on the left. Network analysis showed differences within limbic and basal ganglia circuits, between the posterior association areas and right orbitofrontal areas, and between left dorsolateral frontal and orbitofrontal areas. This combination of preserved perception, with differences in connections between multimodal association areas and limbic/basal ganglia circuits and between limbic and frontal lobes, is consistent with previous clinically based speculations on abnormal brain function in DMS. This work was done by M. Mentis, E. Weinstein, and R. McIntosh.

#### D. UNIT ON BRAIN IMAGING AND COMPUTERS

This unit is responsible for conducting research involving in vivo structural imaging of the human brain in healthy subjects and in the patient groups noted above. Images are obtained using X-ray computer-assisted tomography (CT) or magnetic resonance imaging (MRI). Quantitative volumetric analyses are performed in order to assess differences in volumes of significant brain structures (e.g., ventricles, basal ganglia), and to determine volumetric changes in individuals followed longitudinally. This Unit also conducts research on human in vivo brain phosphorus metabolism using magnetic resonance spectroscopy (MRS). In addition, this Unit conducts research involving the use of multivariate statistical methods and computer computational techniques for analyzing brain functional activity as measured by PET.





## I. THE AGING BRAIN

1. Magnetic resonance imaging and temporal lobe volumes in healthy aging. Temporal and posterior frontal brain volumes were quantified from coronal MRI scans in a cross-sectional study of 30 very healthy men aged 21-92 years. A significant age-related decrease (approximately 1% per decade) of posterior frontal, but not of temporal lobe volume was found. This research was performed by C. DeCarli and B. Horwitz.

2. CSF and subcortical nuclei in healthy aging. Healthy male subjects free of primary brain disease were studied using MRI to determine age differences in the volumes of gray matter nuclei, cerebral ventricles, cerebral brain matter volume and cerebrospinal fluid (CSF). Significant age related atrophy in the lenticular and caudate nuclei was found, as was increased CSF in the elderly subjects. The aging process was found to affect the caudate and lenticular nuclei significantly more than the cerebral hemispheres. This work was performed by D. Murphy and C. DeCarli.

3. Age Differences in Correlations of Cerebral Glucose Metabolic Rates in Normal Adults. The correlation analysis was applied to resting FDG data collected from 15 young (age 21-38 yr) and 17 old (age 65-90 yr) women using a high resolution Scanditronix tomograph. Old women had smaller frontoparietal correlations than young women. This result corroborates our previous correlational analysis applied to data collected with the lower resolution ECAT II tomograph (Horwitz et al., Ann. Neurol., 19, 60-67, 1986). This work was performed by N. Azari and B. Horwitz.

## II. ALZHEIMER DISEASE

1. Longitudinal CT changes in dementia of the Alzheimer type (DAT). The rates of lateral ventricular enlargement and decline in cognitive performance for 11 men and nine women with Dementia of the Alzheimer type (DAT) were determined and compared to the same measures obtained for nine male and eight female age-matched healthy controls. The rate of total lateral ventricle enlargement (cm<sup>3</sup>/year) was significantly different between DAT and healthy controls, and was more specific and sensitive to the diagnosis of DAT than comparison of cross-sectional volumes at final evaluation. The rate of total lateral ventricular enlargement very early in the course of Alzheimer disease was significantly less than the rate of ventricular enlargement after the onset of nonmemory cognitive deficits, but stable for other degrees of dementia severity, which suggests a biphasic process. This work was performed by C. DeCarli and J. Haxby.

2. MRI volumetric changes in men with DAT. A quantitative morphometric MRI study of the brain in DAT was performed to see if cognitive decline in DAT patients was related to brain morphometric changes. DAT men had significantly less cerebral brain matter and significantly larger volumes in every CSF measure (except right and left peripheral CSF) than old healthy men. Men with mild DAT had significantly less cerebral brain matter and significantly larger lateral ventricles than old healthy men. Neuropsychological measures of disease severity in DAT patients were significantly and appropriately correlated to the normalized volumes of cerebral brain matter and right lateral ventricle. This work was performed by D. Murphy, C. DeCarli and B. Horwitz.

3. Discriminant analysis of MRI data in DAT. Temporal and posterior frontal brain volumes were quantified from MRI in a study of 31 male and female patients with DAT and 29 matched healthy controls. Mean MMS scores were in the mild range for the DAT group, but patients with moderate and severe dementia were also included (MMS range 4-28). Although significant mean differences in frontal and temporal lobe brain volumes were found between the DAT group and controls, overlap between groups was found for each individual measure. A multivariate discriminant analysis correctly classified all healthy controls and DAT patients using different discriminant functions according to gender. Cross-validation of the male discriminant function was successful on a second group of mildly demented subjects. These results suggest that discriminant analysis of MRI



volumetric data can add significant diagnostic certainty for those patients with very mild dementia in whom the clinical identification of Alzheimer's disease is unclear. This work was performed by C. DeCarli, D. Murphy, A. McIntosh and B. Horwitz.

4. Magnetic resonance spectroscopy. PET, MRI and magnetic resonance spectroscopy (MRS) were used to study brain glucose (rCMRglc) and  $^{31}\text{P}$  phosphorus metabolism in DAT patients and matched control subjects. PET, MRI, and MRS were all carried out on the same volume of brain matter, from 3.5 - 6.5 cm above the inferior orbitomeatal line. Mean rCMRglc was significantly lower in the DAT group than controls. However, no significant group difference in any phosphorus metabolite concentration/ratio was found, and no significant correlation between any phosphorus metabolite concentration/ratio and severity of dementia, or glucose metabolism was obtained, suggesting that glucose metabolism is reduced early in DAT (reflecting decreased basal synaptic functioning), and is unrelated to a rate-limitation in glucose delivery, abnormal glucose metabolism, or abnormal coupling between oxidation and phosphorylation. Normal or near normal levels of phosphorus metabolites are maintained at all stages of DAT. This work was done by D. Murphy, B. Horwitz, J. Alger, P. Bottomley, C. DeCarli and J. Salerno.

5. Early detection of Alzheimer's disease (AD) using PET and discriminant analysis. A multiple regression/discriminant analysis procedure was applied to resting rCMRglc PET data in mildly/moderately demented AD probable patients and age-matched controls. The results demonstrated that a discriminant function, reflecting functional interactions involving frontal and parietal regions, successfully distinguished AD patients from controls, and identified an AD-like pattern in the first PET scan of the at risk subject (at which time the subject only showed mild memory impairment, was diagnosed with possible AD, and had a normal scan as assessed by traditional analytic methods; at the second evaluation, however, this subject was diagnosed with probable AD, and his scan showed typical AD hypometabolism). The discriminant function was partially validated by applying it to subjects with Down Syndrome with and without dementia. This work was performed by N. Azari, B. Horwitz, K. Pettigrew and J. Haxby.

6. Differences in Patterns of Cerebral Glucose Metabolic Rates in Alzheimer's Patients. Correlation analysis was applied to resting normalized rCMRglc data collected using the Scanditronix PC1024-7B tomograph from 19 patients (12 men, 7 women) with mild-to-moderate dementia of the Alzheimer type (DAT) (ages: 52-81 yr) and 22 healthy, age-matched controls (ages: 53-75 yr). The DAT group had fewer positive significant correlations in the whole brain than controls. Values in the DAT group generally were smaller than in controls for frontoparietal, frontal-sensorimotor, frontal-limbic and sensorimotor-parietal correlations. The results corroborate our previous work using a low resolution scanner showing decreased frontoparietal associations in mild/moderate DAT patients in the 'resting' state (Horwitz et al., Brain Res. 407, 294-306, 1987), and demonstrate decreased interactions between regions which modulate attention. This research was done by N. Azari and B. Horwitz.

### III. NETWORK ANALYSIS

1. Functional coupling during visual processing. Correlational analysis was performed on normalized rCBF values, obtained by PET with  $[15\text{-O}]\text{-labeled}$  water, to examine functional interactions among brain regions in posterior neocortex in young men during two 2-choice, match-to-sample visual tasks: face discrimination (FD), and dot localization with rotation (DL). Although both tasks activated lateral occipital and occipitotemporal cortex bilaterally, correlational analysis revealed that during FD, occipital and occipitotemporal activations correlated significantly, but only in the right hemisphere. During DL, occipital and superior parietal activations correlated significantly, but again, only in the right hemisphere. These results support the view that FD and



DL processing are carried out to a greater degree by the right posterior hemisphere. This work was performed by B. Horwitz, J. Haxby, C. Grady, and M. Schapiro.

## 2. Functional interactions in corpus callosotomized rat brain.

Correlation coefficients among normalized rCMRglc for 97 brain regions and their contralateral homologues were examined in rats that had undergone corpus callosotomy and in sham-operated controls. Correlation coefficients between left-right region pairs were more often lower than higher in callosotomized animals, indicating that the corpus callosum (CC) is important for functional interactions of these structures. This work was performed by T. Soncrant, S. Sato and B. Horwitz.

## 3. Functional interactions in rat brain following nucleus basalis

magnocellularis ablation (nbml). Unilateral destruction of the nbm in rats reduces cholinergic inputs to the ipsilateral frontoparietal neocortex and disrupts behavior. Correlational analysis of rCMRglc was performed on 2 groups of 16 young rats at 2 wk after ablation of the right nbm with ibotenate or sham surgery. Most correlations between cholinergic nuclei (medial septum and diagonal band) and right but not left frontoparietal cortical regions were larger in lesioned rats, as were those between most frontoparietal region pairs. This work was performed by T. Soncrant, Y. Lamour and B. Horwitz.

## 4. Computer simulation model for correlational analysis of regional

cerebral blood flow (rCBF) values. A computer simulation model was devised in order to provide a partial validation for correlation analysis as applied to rCBF data. Because the underlying pattern of functional couplings in the model is specified, these simulations demonstrate that the change in the correlation coefficient between normalized rCBF values reflects the change in the corresponding functional coupling. An example of the use of the simulation method showed how rCBF activations during a task (relative to a control state) could be bilateral in the brain, even though non-zero within-hemisphere functional couplings were found only in one hemisphere. This work was carried out by B. Horwitz.

## 5. Network analysis of cortical visual pathways mapped with PET.

A network analysis was performed on data obtained from a PET study that examined both the changes in regional cerebral blood flow (rCBF) and interregional correlations among human cortical areas during performance of a face matching (object vision) and dot-location matching (spatial vision) task (see 12 above). Brain areas for the anatomical network were based on human and primate studies of visual processing. Interactions among selected regions were quantified using structural equation modeling. The functional network for the right hemisphere showed that in the object vision task, dominant path influences were among occipitotemporal areas while in the spatial vision task, occipitoparietal interactions were stronger. There were strong interactions between dorsal and ventral pathways in both networks. This work was performed by A. McIntosh and B. Horwitz.

## E. UNIT ON NEUROPSYCHOLOGY

### 1. Activation and localization of cortical visual pathways.

Studies of regional cerebral blood flow, as measured with  $^{15}\text{O}$  and positron emission tomography (PET), during the performance of spatial and object vision tasks in healthy men, demonstrated two anatomically distinct and functionally specialized pathways, the locations of which were not absolutely predicted from studies in nonhuman primates. Young and old adults had rCBF increases with similar magnitudes and spatial distributions. Patients with mild Alzheimer's disease had rCBF increases during the object vision task that had the same neuroanatomical location and magnitude as that seen in controls, even though this regions demonstrated reduced rCBF. Patients with Alzheimer's disease additionally demonstrated activation in prefrontal regions not activated in healthy controls. Preserved capacity to increase rCBF suggests that the neural systems associated with recruiting association areas for cognitive processes are not impaired when



the areas themselves are. The additional activation of frontal areas suggests a compensatory mechanism, reflecting the increased attentional load associated with face matching performance in patients with Alzheimer's disease. This work was done by J.V. Haxby, C.L. Grady, B. Horwitz, M.B. Schapiro, M. Mishkin, L. Ungerleider, P. Herscovitch, and R. Carson.

2. Longitudinal course of neuropsychological impairment in DAT. Longitudinal study of patients with DAT for as long as five years has shown that there can be an initial plateau phase during which only memory is impaired, but decline on other neuropsychological functions is not observed. Afterwards, neuropsychological decline on nonmemory cognitive abilities is remarkably linear. The rate of decline varies markedly among patients, but the rate for an individual is quite constant. Consequently, future rate of decline can be predicted, based on longitudinal neuropsychological evaluations. After memory impairment, the first neuropsychological functions to demonstrate significant deficits in patients with mild DAT are attention, planning and foresight, and abstract reasoning. Patterns of nonmemory neuropsychological impairments tend to persist over time, despite worsening severity of overall dementia. For example, patients with disproportionate verbal impairment relative to visuospatial impairment tend to demonstrate the same neuropsychological discrepancy at follow-up. This work was done by J.V. Haxby and C.L. Grady.

3. Metabolic and neuropsychological patterns are correlated in DAT. In patients with moderate Alzheimer's disease, the relative disproportion of language vis-a-vis visuospatial impairments was significantly correlated with right-left asymmetry of regional cerebral metabolic rates for glucose (rCMRglc). Moreover, discrepancies between calculations or immediate visuospatial memory span, on the one hand, and attention or verbal fluency, on the other, correlated significantly with metabolic discrepancies between parietal and frontal association cortices. In mildly demented patients, metabolic patterns were not correlated with nonmemory neuropsychological patterns. These patients had no significant impairments of nonmemory language and visuospatial functions with significant abnormalities of neocortical metabolism. Follow-up studies demonstrated significant deterioration of nonmemory neuropsychological functions, which correlated with right-left metabolic asymmetries. These findings confirm that neocortical metabolic changes precede the development of demonstrable neocortically mediated neuropsychological impairments in early DAT. It appears that the brain is capable of compensating for these early physiological changes to maintain premorbid neuropsychological function. This work was done by J.V. Haxby and C.L. Grady.

4. Selective attention in healthy aging and DAT Cognitive studies of selective and shifting attention to visual features in compound stimuli identified two dissociated underlying processes, one driven by intention and the other requiring priming with practice. Patients with mild dementia of the Alzheimer type were found to have disproportionate impairments on tests of selective and divided attention to visual features. In a separate study, patients with DAT were found to have impaired selective attention to spatial location, such that they were less efficient at responding to stimuli in unattended locations. This impairment was correlated with superior parietal lobule rCMRglc, providing evidence for the neuroanatomical basis for this aspect of the attentional impairment in DAT. A study of selective and divided attention to sensory modality (visual and auditory) found that aging was associated with no change in the ability to attend selectively to one channel and increasing difficulty shifting attention from one sensory channel to the other. By contrast, patients with DAT were found to have impairment of both selective and shifting attention, although the impairment of shifting attention was more marked. This work was done by J.V. Haxby and A. Berardi in collaboration with R. Parasuraman and P. Greenwood of Catholic University.

5. Long-term recognition memory. Long term recognition memory for faces was associated with increased rCBF in right prefrontal cortex, left anterior cingulate and right inferior parietal cortex, but with decreased rCBF in primary visual cortex and in bilateral areas of the superior and midtemporal cortex of





young adults. The rostral superior temporal sulcus is a high order visual area with face-selective cells in the monkey. Decreased activity in this area may reflect signal familiarity. This work was done by J. V. Haxby and C. L. Grady.

6. Changes in rCBF with increased task difficulty in working memory. Working memory for faces in young adults was associated with rCBF increases in prefrontal cortex, whose laterality varied with length of retention interval. At shorter intervals (1 to 11 sec), prefrontal rCBF was greater in right hemisphere, whereas as at longer intervals (16 and 21 sec), it was greater in the left hemisphere. Subjects reported that mental image of face faded with interval prolongation. These results suggest that working memory for a face may shift from the visual image in the right prefrontal cortex to the analytical representation in the left. This work was done by J. V. Haxby and colleagues.

#### F. UNIT ON PHARMACOLOGY AND PHARMACOKINETICS

1. Arecoline treatment of human subjects with Alzheimer's disease. Arecoline was administered intravenously to 15 demented elderly subjects, and plasma pharmacokinetics were measured. Steady-state plasma levels were rapidly achieved and, after termination of the infusion, arecoline was cleared from plasma with a half-life of less than five minutes. Administered by chronic, continuous intravenous infusion, arecoline improved memory performance in patients with Alzheimer's disease, at doses producing no side-effects. A plasma drug level corresponding to cognitive optimization was identified. Hence, arecoline and related drugs may be effective for palliation of Alzheimer's disease. This work was conducted by T. Soncrant, K. Raffaele, and S. Asthana.

2. Physostigmine treatment of patients with Alzheimer's disease. Physostigmine, a cholinesterase inhibitor, was administered to 9 patients with mild-moderate dementia. Steady-state drug levels were achieved by continuous infusion. Modest cognitive improvement occurred in some subjects; adverse effects were frequent. Specific muscarinic agonists may be more effective than are cholinesterase inhibitors in the palliation of cognitive symptoms of Alzheimer's disease. Conducted by S. Asthana, T. Soncrant, K. Raffaele.

3. Age-related cognitive and psychomotor responses to haloperidol. Young and elderly subjects received haloperidol intravenously. Young subjects showed greater akathisia and sedation, and had higher plasma prolactin levels after haloperidol administration. The results suggest a reduced effect of brain dopamine receptor blockade on cognitive and motor function in aged humans. Conducted by J. Kelly and J. Kaye.

#### G. SECTION ON NEUROCHEMISTRY AND BRAIN TRANSPORT

##### I. FUNCTION AND STRUCTURE OF BLOOD-NERVE AND BLOOD-BRAIN BARRIERS

1. Secondary ion mass spectrometry method to map element distribution in Alzheimer's disease brain. A high resolution secondary ion mass spectrometric (SIMS) method was developed to map the distribution of aluminum and other elements in unfixed, unstained Alzheimer's disease brain. Signal intensity for aluminum was shown to be proportional to concentration with a limit of detection in the part-per-million range. Morphologic identity was established with post staining. The results demonstrate that SIMS will provide a highly sensitive and accurate tool to map element distribution in postmortem Alzheimer's disease brain. This work was done by Q. Deng, D. Brady and Q. Smith in collaboration with researchers at the National Institute of Standards and Technology.

2. Improving brain drug delivery through carrier-mediated transport. Rat brain perfusion studies identified six anticancer drugs that are taken up into brain via the large neutral amino acid carrier of the blood-brain barrier. One agent, D,L-NAM, exhibited markedly high affinity for the carrier ( $K_m \leq 0.2 \mu M$ ) and was taken up into brain 20-40 times more rapidly than its clinical analogue, L-melphalan. Uptake was also elevated to brain tumors. The results demonstrate the feasibility of improving drug delivery to brain through the design and



synthesis of high affinity analogs to the cerebrovascular nutrient carriers. This work was done by Q. Smith and Y. Kohmo.

3. Identification of the basic amino acid transporter of the blood-brain barrier. Nutrient uptake into brain is mediated by various carrier- and receptor-mediated systems. The basic amino acid transporter of the rat blood-brain barrier was identified in brain perfusion studies and shown to be identical to System  $y^+$ , the sodium-independent cationic amino acid transporter. The mRNA for System  $y^+$  was shown to be present in brain microvessels at a level that exceeded that in whole brain by >30 fold. The results demonstrate that System  $y^+$  is the basic amino acid transporter of the blood-brain barrier and is densely expressed in cerebral capillaries. This work was done by J. Stoll, K. Wadhvani and Q. Smith.

4. Polymerase chain reaction titration method for quantification of mRNA. A modified polymerase chain reaction titration (PATTT) method was developed to accurately quantify mRNA levels in cells and tissues. The method overcomes problems in standard polymerase chain reaction experiments and is capable of accurately detecting a 2-fold change in tissue mRNA. The method will be a valuable tool in studying gene expression both in vivo and in vitro. This work was done by K. Wadhvani.

5. Blood-nerve barrier permeability and nerve vascular volume during aging. The blood-nerve barrier permeability to [ $^{14}$ C]sucrose was examined in Fischer-344 rats using an intravenous injection technique and found to remain stable between 3 mo and 30 mo of life. Morphometric measurements of nerve vascular surface area and blood volume also did not change. The results suggest that the integrity of the BNB is maintained with age in the rat. This work was conducted by K. Wadhvani.

6. Saturable transport of amino acids and metals across the blood-nerve barrier. Nerve, like brain, requires certain metals and nutrients for normal metabolism and function. Many of these solutes, because of their poor lipid solubility, only poorly cross barrier membranes by passive diffusion. K. Wadhvani and Q. Smith demonstrated that both essential neutral and basic amino acids, as well as manganese, are transported into nerve by saturable carriers at the blood-nerve barrier. The transporters, for the most part, had properties similar to those at the blood-brain barrier and other tissues. The results demonstrate that while the blood nerve barrier differs in many respects from the blood-brain barrier, critical functions, such as facilitated uptake of essential nutrients and metals, are maintained.

7. Calcium transport across blood-brain and blood-nerve barriers. Although calcium concentrations are regulated within cerebrospinal fluid, studies with radiolabeled calcium in awake rats fed diets with low, normal or high calcium indicated no change in the permeability-surface area for calcium at blood vessels of brain and nerve. This means that cerebrospinal fluid calcium must be regulated by transport at the choroid plexus, and that calcium content of the nerve is not regulated at the steady state. This work was done by K. Wadhvani and V. Murphy.

## II. DRUG DEVELOPMENT AND DELIVERY TO CENTRAL NERVOUS SYSTEM

1. Chemical modification of water soluble drugs for treating brain tumors. The alkylating anticancer agent, chlorambucil, normally enters the brain very slowly and is ineffective against brain but not peripheral tumors. Drug development studies were undertaken to modify this agent to overcome these pharmacokinetic defects due in part to the blood-brain barrier. One drug developed was a tertiary butyl ester of chlorambucil, which was found to have optimal brain uptake (due to a high lipid solubility) and a long plasma half-life (due to steric hindrance of plasma esterases), while retaining significant alkylating activity. It was shown to be active against intracerebral tumor implants in rodents, and against human brain tumors in vitro which were insensitive to chlorambucil (as its lipid solubility enhances its cell entry).



This and similar chlorambucil derivatives also were shown to be effective in treating tumors of the lymphatic system, breast and ovaries. This work was done by N. Greig.

2. Development of anticholinergic agents for treating Alzheimer disease. The loss of cortically-projecting basal forebrain cholinergic neurons characterizes the brain in Alzheimer disease and may contribute to memory deficits in that disorder. Physostigmine, an anticholinesterase, has been used to treat Alzheimer disease with limited success, possibly because of its short plasma half-life and lack of specificity for brain acetylcholinesterase. A large number of physostigmine derivatives were synthesized, with substitutions in the carbamoyl and N(1) positions, and were shown to have prolonged plasma half-lives and anticholinergic actions, and increased specificity for acetyl- as compared to butyrylcholinesterase. Some of these compounds (eg pheneserine) have been shown to attenuate scopolamine-induced learning impairment of rats. This work was done by N. Greig and colleagues.

3. Development of therapeutics for treatment of drug abuse. Cocaine, a major drug of abuse, is thought to act by inhibiting reuptake of biogenic amines; its ability to inhibit dopamine uptake is thought to cause its addictive action. To see whether inhibitors of dopamine reuptake could act as a cocaine antagonist, the high affinity inhibitor GBR 12909 was studied and was shown to be capable of inhibiting the dopamine transporter in rat brain striatal membranes, as well as the ability to elevate extracellular dopamine levels. These data suggest that tight-binding dopamine reuptake inhibitors may be useful as cocaine antagonists. This work was done by N. Greig and R. Rothman.

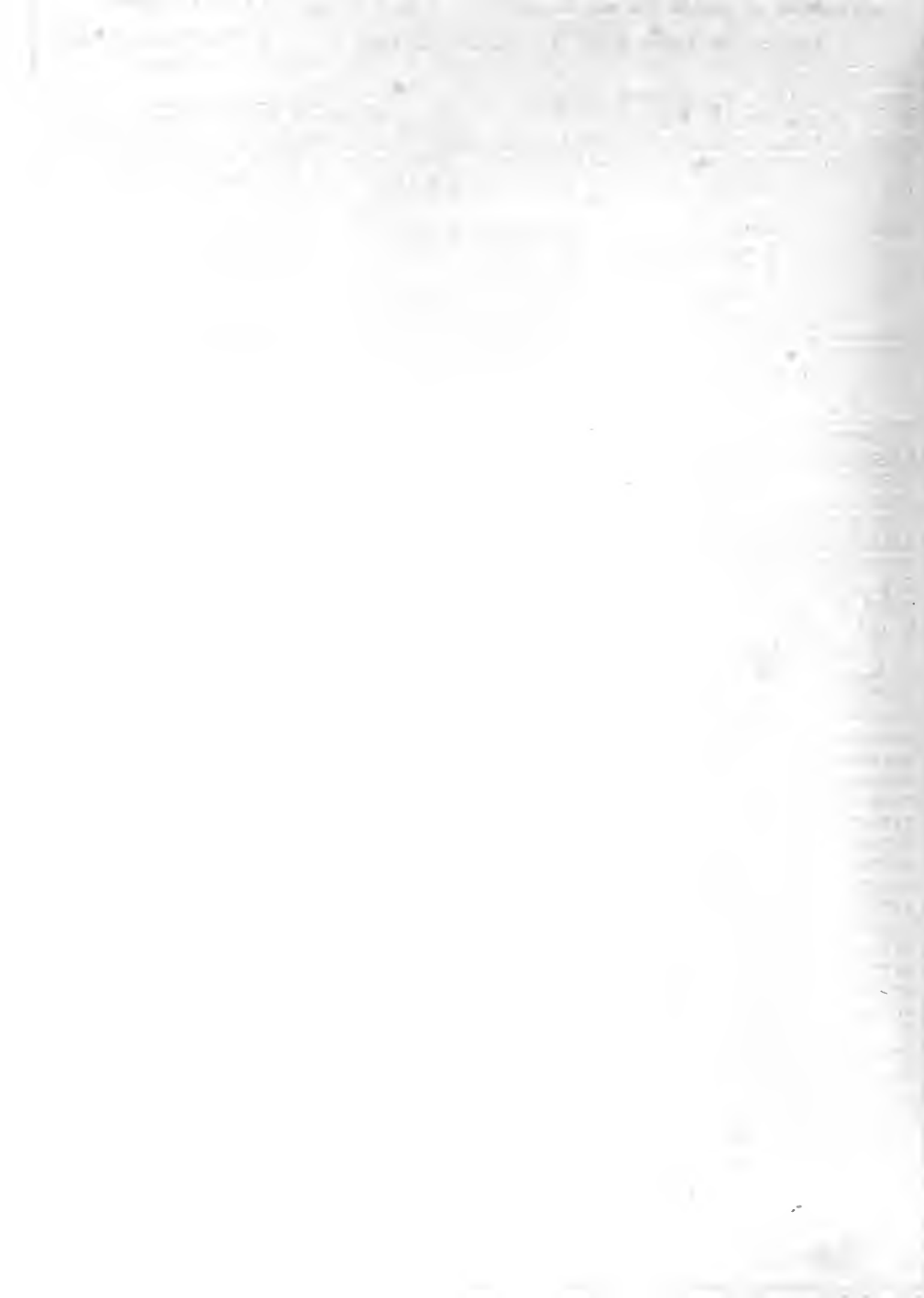
4. Therapeutics for treatment of brain sequestered human immunodeficiency disease. The brain is an early site of HIV infection, and encephalopathy frequently becomes apparent. A variety of dideoxynucleoside analogs have been developed to inhibit reverse transcriptase processes of HIV in the brain, including 3'-azido-2'-3'-dideoxythymidine (AZT) and 2'-3'-dideoxycytidine (DDC); these analogs can be metabolized in mammalian cells and inhibit HIV replication at doses that are not antiproliferative. However, they are water soluble and have restricted brain uptake. Lipophilic derivatives of DDC and AZT have been developed and were shown to attain high therapeutic levels in brain in animal studies. This work was done by N. Greig and P. Torrence.

5. Liposome delivery of drugs to the brain. Liposomes which were targeted to the transferrin receptor on cerebral capillaries were shown to accumulate selectively in the brain of rats. This technology should allow increased delivery of peptides and labile water-soluble agents to the brain, by enclosing them within liposomes which can accumulate in the capillary vascular bed and form a source for slow release. This work was done by N. Greig and S. Rapoport.

6. Entry of positively-charged plasma protein into brain. Quantitative measurements in rats demonstrated that positively charged bovine serum albumin in blood penetrates brain capillaries much more rapidly than the native protein and in measurable quantities. Such positively charged proteins can be used as carriers for covalently-bound peptides and other drugs across the blood brain barrier. This work was done by S. Rapoport.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AG 00407-02 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the <u>Neuroanatomy and Neuropathology of Aging Primate Brain</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal PI: D. Brady Senior Staff Fellow LN, NIA		
Others: J. Stoll Senior Staff Fellow LN, NIA K. Chandrasekaran Visiting Scientist LN, NIA A. Balbo Biologist LN, NIA R. Fukuyama Visiting Fellow LN, NIA C. DeCarli Senior Medical Officer LN, NIA E. Mufson Professor Rush Med		
COOPERATING UNITS (if any) Division of Neurosciences, Rush-St. Luke's Medical Center, Chicago, IL;		
LAB/BRANCH Laboratory of Neurosciences		
SECTION None		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input checked="" type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Similar topography, morphology and pattern of fiber staining of <u>nicotinamide adenine dinucleotide-diaphorase (NADPH-d)</u> , <u>parvalbumin</u> , <u>neuropeptide Y</u> , <u>somatostatin</u> and <u>SMI-32</u> containing structures were demonstrated in the <u>amygdala</u> of the <u>monkey</u> , <u>gorilla</u> , <u>chimpanzee</u> and normal <u>aged human</u> brain. These similarities support the usefulness of experimental paradigms comparing these neurochemical systems in non-human and human primates. While the primate basolateral amygdaloid division developed phylogenetically in tandem with the expansion of neocortical association areas, the greatest increase was observed in humans. Because the lateral amygdaloid nucleus maintains direct synaptic contact with cerebral cortical association areas, and harbors a distinct population of parvalbumin and SMI-32 containing neurons, it was used to test the selective vulnerability hypothesis in <u>Alzheimer's disease</u> (AD). The lateral nucleus showed up to a 50% decrease in parvalbumin <u>neurons</u> . These results suggest that connectivity of a specific cytoarchitectural area features in the pathogenesis of AD. A molecular biology approach was also used to test selective vulnerability. Neuropathologic analysis of AD patients identified clinically with <u>leukoencephalopathy</u> did not support a direct vascular role in the etiology of the disease. Amyloid staining revealed severe cerebral <u>amyloid angiopathy</u> without involvement of white matter vessels. Thus, the increased amyloid burden in the cerebral vasculature does not appear to account for clinically observed leukoencephalopathy in AD patients.		





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 AG 00403-08 LN
<b>PERIOD COVERED</b> October 1, 1992 to September 30, 1993		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Genetics and Nongenetic Factors in Alzheimer's Disease)</b>		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal PI:</b>		
Mark Schapiro	Chief, Section BAD	LN, NIA
<b>Others:</b>		
C. Grady	Chief, PET Unit	LN, NIA
K. D. Pettigrew	Statistician	MHIRP, NIMH
Zheng Wu	Visiting Associate	LN, NIA
Beverly White	Medical Res Officer	LCB, NIDDK
Katherine K. Sanford	Chief	LCMB, NCI
Ram Parshad	Professor	Howard Univ
<b>COOPERATING UNITS (if any)</b> CRND Univ., Toronto; Dept. Pathology, Howard Univ.; Twin Registry, NAS; LCNSS, NINDS; MHIRP, NIMH; LCMB, NCI; LCB, NIDDK		
<b>LAB/BRANCH</b> Laboratory of Neurosciences		
<b>SECTION</b> Brain Aging and Dementia		
<b>INSTITUTE AND LOCATION</b> NIA, NIH Bethesda, Maryland 20892		
<b>TOTAL STAFF YEARS:</b> 3.0	<b>PROFESSIONAL:</b> 1.5	<b>OTHER:</b> 1.5
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b> <u>Pedigrees</u> were constructed from <u>family histories</u> of patients participating in a <u>dementia</u> program to examine the <u>genetic</u> basis of <u>Alzheimer's disease</u> . Experimental protocols were developed for comparing <u>phenotypes</u> and <u>genotypes</u> of individuals with duplications of parts of <u>chromosome 21</u> , to identify genes contributing to mental retardation and dementia. <u>Buffy coats</u> from blood of <u>first degree relatives</u> with dementia of the Alzheimer type were injected into hamsters to test <u>transmissibility</u> in Alzheimer's disease. In animals surviving up to 301 days after inoculation, no evidence of brain disease was present, arguing against transmissibility.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH  
SERVICE

PROJECT NUMBER

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00140-10 LN

## PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the  
Cerebrospinal Fluid Chemistry in Aging and Dementia)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)

PI:	M. B. Schapiro	Chief, SBAD	LN, NIA
	U. Shetty	Visiting Associate	LN, NIA

OTHERS:	H. Holloway	Biologist	LN, NIA
	J. R. Attack	Research Scientist	Merck
	I. Hanin	Director	Dept. Pharm, Loyola Univ
	M. F. Beal	Neurologist	Mass Gen. Hosp

## COOPERATING UNITS (if any)

Dept of Pharmacology and Experimental Therapeutics, Loyola Univ.; Dept of  
Neurology, MA General Hospital, Boston, Ma; Merck, Sharpe and Dohme, England.

## LAB/BRANCH

Laboratory of Neurosciences

## SECTION

Brain Aging and Dementia/Cerebral Physiology and Metabolism

## INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland, 20892

## TOTAL STAFF YEARS:

1.4

## PROFESSIONAL:

1.4

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human      ☐ (b) Human      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In cerebrospinal fluid, acetylcholinesterase activity and somatostatin and neuropeptide Y concentrations did not differ from control values in either young or old Down syndrome patients. Analysis of lumbar cerebrospinal fluid fractions in healthy young and old subjects indicated no anterior-posterior gradients for somatostatin and neuropeptide Y, but higher acetylcholinesterase in caudal as compared to rostral fractions in younger subjects, indicative of sources for this enzyme from the spinal cord. Acetylcholinesterase gradients in cerebrospinal fluid were absent in old healthy subjects, who had higher mean activity overall. CSF production rates and caudorostral gradients of CSF protein were normal in DS adults.

Cerebrospinal fluid and plasma myo-inositol levels in Down's syndrome patients were determined by a newly developed mass spectrometric assay. Myo-inositol levels in cerebrospinal fluid in Down's syndrome were increased significantly compared to normals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00126-13 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Brain Function in Aging and Dementia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal PI: M. Schapiro Chief, BADS LN, NIA C. Grady Chief, Unit on PET LN, NIA B. Horwitz Chief, Unit on Brain Imaging LN, NIA and Computers J. Haxby Chief, Unit on Neuropsychology LN, NIA J. Salerno Guest Worker LN, NIA D. Murphy Visiting Associate LN, NIA		
COOPERATING UNITS (if any) Child Psychiatry Branch, NIMH; Department of Nuclear Medicine, CC; Laboratory of Neuropsychology, NIMH		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Brain Aging and Dementia		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Age-related differences in metabolism and cerebral blood flow (rCBF), as measured by positron emission tomography (PET), were enhanced in <u>frontal cortex</u> when <u>young</u> and <u>old</u> subjects received <u>sensory activation</u> . Dorsal vs. ventral patterns of rCBF activation during <u>face and location matching</u> were similar in young and old subjects, but young subjects had more activation of occipital cortex and older subjects had more frontal activation. <u>Activation</u> of rCBF in <u>occipitotemporal</u> <u>cortex</u> was equivalent during <u>face perception</u> in patients with dementia of the Alzheimer type (DAT) and controls; patients also had frontal activation. The activation results are consistent with the hypothesis of <u>reversible synaptic</u> <u>dysfunction</u> in early <u>Alzheimer's disease (AD)</u> . A patient with isolated memory impairment and family history for <u>autosomal dominant AD</u> was studied with PET twice and found to have normal glucose metabolism with routine analysis but both scans were abnormal using a <u>discriminant function analysis</u> . A patient with autopsy proven <u>Parkinson's disease</u> had a metabolic pattern indistinguishable from that seen in DAT. Regional densities of <u>neurofibrillary tangles</u> , but not of <u>senile plaques</u> <u>in postmortem brain</u> , were correlated with metabolic reductions in 5 AD patients who were scanned prior to death. A two-stage hypothesis of dementia in <u>Down syndrome</u> was suggested by longitudinal patterns of neuropsychological decline and measures of <u>brain atrophy</u> . Subjects with <u>Turner syndrome (45,X)</u> , including mosaics, had reduced volume of the <u>hippocampus</u> , lower parietal metabolism and impairment of visuospatial abilities.		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00404-07 LN

## PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the  
Functional Interactions Among Brain Regions in Aging and Dementia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI:	B. Horwitz	Chief, Unit on Brain Imaging & Computers	LN, NIA
	N. Azari	NRC Fellow	LN, NIA
	A.R. McIntosh	NSERC Fellow	LN, NIA
Others:	M. Mentis	Visiting Associate	LN, NIA
	C. Grady	Research Psychologist	LN, NIA
	M. Schapiro	Chief, BADS	LN, NIA

## COOPERATING UNITS (if any)

LN, NIMH; CPB, NIMH; DASR, NIMH

## LAB/BRANCH

Laboratory of Neurosciences

## SECTION

Brain Aging and Dementia

## INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

3.2

## PROFESSIONAL:

3.0

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human      ☐ (b) Human      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A correlation method was developed to examine functional interactions between brain regions, by correlating either regional cerebral metabolic rates for glucose or regional cerebral blood flows, as determined by positron emission tomography (PET) in humans. There was a loss of frontal-parietal functional associations with age, and a further reduction in patients with dementia of the Alzheimer type (DAT), suggesting fewer corticocortical connections. In patients with well controlled hypertension, correlations involving regions in the vascular watershed areas were significantly reduced. In humans in whom regional cerebral blood flow (rCBF) was measured with PET during two visual processing tasks, correlations among visual brain areas were significant in the right but not the left hemisphere, suggesting a more important role for the right hemisphere. A systems-level neural network-model, fitted to rCBF PET data, permitted determination of the brain regions and their interactions that were involved in two visual processing tasks. A computer simulation model was developed to explore the neurobiological substrates of observed correlational patterns. A multiple regression/discriminant analysis involving PET regional interdependencies distinguished DAT patients from controls. In young adults with Down syndrome, PET values in language areas could be used in a discriminant function to distinguish their PET scans from those of controls. A multiple regression/discriminant analysis applied to PET scans of patients with Obsessive-Compulsive Disorder obtained before and after pharmacotherapy allowed discrimination between patients who responded to drug from those who did not.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 AG 00130-10 LN
<b>PERIOD COVERED</b> October 1, 1992 to September 30, 1993		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Neuropsychological Function in Aging and Dementia)</b>		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)</b>		
P.I.:	J.V. Haxby                      Unit Chief, Neuropsychology C.L. Grady                      Research Psychologist G. Alexander                   Unit Chief (7/93)	LN, NIA LN, NIA LN, NIA
Others:	M. Kurkjian                   Staff Fellow J. Maisog                      Staff Fellow M. Mishkin                   Chief, LNP L.G. Ungerleider              Research Psychologist	LN, NIA LN, NIA LNP, NIMH LNP, NIMH
<b>COOPERATING UNITS (if any)</b> Laboratory of Neuropsychology, NIMH Department of Psychology, Catholic University		
<b>LAB/BRANCH</b> Laboratory of Neurosciences		
<b>SECTION</b> Brain Aging and Dementia Section		
<b>INSTITUTE AND LOCATION</b> NIA, NIH, Bethesda, Maryland 20892		
<b>TOTAL STAFF YEARS:</b> 4.0	<b>PROFESSIONAL:</b> 2.0	<b>OTHER:</b> 2.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b>  Cortical systems that participate in <u>object</u> and <u>spatial vision</u> and in working and long-term <u>visual memory</u> were investigated in healthy young men by measuring <u>regional cerebral blood flow (rCBF)</u> with <u>positron emission tomography (PET)</u> and <u>H215O</u> . The results identified dorsolateral occipital and superior parietal areas activated more by spatial visual processing, and ventral occipital and occipitotemporal areas activated more by object discrimination. <u>Old subjects</u> demonstrated rCBF activations in the same regions as did <u>young subjects</u> , but also demonstrated activation of ventral areas during spatial vision and dorsal areas during object vision, suggesting less functional separation of these visual systems. Patients with <u>dementia of the Alzheimer type (DAT)</u> demonstrated normal percent baseline activation of rCBF in occipitotemporal cortex during object vision, suggesting preserved capacity to recruit this area for perceptual processing. Memory-related modulations of cortical rCBF were found in anterior temporal and prefrontal cortex. <u>Neuropsychological decline</u> in very early Alzheimer's disease was found to have an early plateau phase followed by steady, linear decline. Rate of cognitive decline was significantly correlated with rate of brain tissue loss, as measured with serial CT scans, and with rate of worsening abnormality of resting state <u>regional cerebral metabolic rates for glucose (rCMRGlc)</u> as measured by PET and <u>18F-Fluorodeoxyglucose</u> . Cognitive studies of complex attention, the first nonmemory function to demonstrate impairment in early DAT, identified three similar impairments of shifting or divided attention. Older <u>Down syndrome adults</u> perform worse on mental abilities tests than do younger subjects. <u>Immediate memory</u> and <u>language</u> function are less affected by age in Down syndrome than are <u>long-term memory</u> and <u>visuospatial function</u> .		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 AG 00132-09 LN
<b>PERIOD COVERED</b> October 1, 1992 to September 30, 1993		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Brain Anatomy in Aging and Dementia)</b>		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)</b>		
C. DeCarli	Senior Staff Fellow	LN, NIA
D. Murphy	Visiting Scientist	LN, NIA
B. Horwitz	Chief, Unit on Brain Imaging & Computers	LN, NIA
<b>Others:</b>		
M. Schapiro	Chief, BADS	LN, NIA
S.I. Rapoport	Chief	LN, NIA
M. Mentis	Visiting Associate	LN, NIA
<b>COOPERATING UNITS (if any)</b> LNC, NIMH; NIS & DIR, NINDS; CC Radiology; General Electric Co., Schnectady, NY.		
<b>LAB/BRANCH</b> Laboratory of Neurosciences		
<b>SECTION</b> Brain Aging and Dementia		
<b>INSTITUTE AND LOCATION</b> NIA, NIH, Bethesda, MD 20892		
<b>TOTAL STAFF YEARS:</b> 1.0	<b>PROFESSIONAL:</b> 1.0	<b>OTHER:</b> 0
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b> Age-related <u>subcortical gray matter</u> atrophy, <u>ventricular dilatation</u> , and the expansion of peripheral cerebrospinal fluid (CSF) volume was found in healthy men using <u>magnetic resonance imaging</u> (MRI), employing a <u>threshold technique</u> which allows quantification of brain matter and CSF volume changes. MRI showed a significant age related decrease of <u>posterior frontal</u> , but not of <u>temporal lobe</u> volume in healthy men. Men with mild DAT had significantly less <u>brain matter</u> than did healthy age-matched controls. <u>Discriminant analysis</u> of volumetric MRI images allowed complete separation between DAT patients and healthy age- and sex-matched controls, including validation on a separate group of mildly demented patients. Mean volumes of both the lateral ventricles were significantly larger in the neurologically and cognitively normal <u>hypertensives</u> as compared to controls using MRI, suggesting that <u>longstanding hypertension</u> results in greater ventricular enlargement than would be expected on the basis of age alone. Volumetric analysis of MRI images in <u>Turner's syndrome</u> (TS) revealed smaller <u>hippocampi</u> , <u>basal ganglia</u> and <u>parieto-occipital</u> brain matter than matched controls. Hippocampal volumes and memory test scores were reduced independently of 'X chromosome dosage' in all TS subjects, suggesting a role for sex steroids in development and death of hippocampal neurons. <sup>31</sup> <u>P spectra</u> , multisection <u>hydrogen images</u> (to quantitate <u>cerebral atrophy</u> ), and fluorodeoxyglucose <u>positron emission tomographic</u> (PET) scans were obtained from DAT patients and healthy age-matched controls. <u>Phosphorus metabolite concentrations</u> did not differ between patients and controls; <u>glucose metabolic rates</u> were reduced in DAT patients relative to controls, suggesting that disturbances in cellular phosphate energy reserves and membrane phosphoester metabolite levels do not play a major role in the neuropathology of DAT, at least in bulk regions of the brain.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AG 00405-06 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the New Investigations in Aging and Dementia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal		
PI:	M. Schapiro	Chief, BADS LN, NIA
	C. DeCarli	Senior Staff Fellow LN, NIA
	D. Murphy	Visiting Associate LN, NIA
	J. Salerno	Senior Staff Fellow LN, NIA
	M. Hertzman	Professor Psychiatry G. W. Univ.
	S. Rapoport	Chief, LN LN, NIA
OTHERS: R. Hagerman Professor of Pediatrics Univ. of Colorado		
COOPERATING UNITS (if any) G. W. Univ., Dept. Psychiatry; School of Medicine, Univ. of Colorado, Laboratory Chemical Biology, NIDDK; Division of Neurology, Dept. of Medicine, Duke Univ.		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Brain Aging and Dementia Section		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input checked="" type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In using the isotope <u>fluoro-18-deoxyglucose</u> with <u>positron emission tomography</u> , we found that <u>cerebral glucose utilization</u> does not change with advancing <u>age</u> in healthy males, but changes are found in patients with <u>Alzheimer's disease</u> and <u>Down</u> <u>syndrome</u> . Three new protocols were introduced to evaluate these findings and determine their <u>specificity</u> . Studies are underway in <u>multi-infarct dementia</u> , the second leading cause of dementia; <u>major depressive disorder</u> with and without cognitive impairment; and <u>fragile-X syndrome</u> . The role of the <u>dopaminergic</u> system in <u>normal aging</u> , <u>Alzheimer's disease</u> with and without <u>extrapyramidal</u> signs, and <u>familial inverted chorea</u> will be explored with 6-[18-F]-fluoro-L-Dopa and positron emission tomography (PET).		
This project has been terminated.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH  
SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00406-03 LN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Mechanisms for Alzheimer Disease)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI: S. Rapoport Chief, LN LN, NIA

OTHERS: M. Schapiro Chief, BADS LN, NIA  
C. Grady Chief, PET Unit LN, NIA  
J. Maisog Medical Staff Fellow LN, NIA  
T. Zeffiro Senior Staff Fellow LN, NIA  
N. Gershfeld Senior Scientist LPB, NIAMS  
L. Ginsberg Visiting Associate LPB, NIAMS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☐ (b) Human ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Comparative anatomic data suggest that several systems of brain regions underwent selective expansion or differentiation during primate evolution, according to the principle of 'integrated phylogeny'. This involved expansion of the neocortex. Certain human neurodegenerative diseases, including Alzheimer disease, affect such systems, suggesting that they are 'phylogenetic diseases' and that the genetic changes that promoted integrated phylogeny are related to the genetics of these diseases. Studies of monozygotic twins discordant and discordant for Alzheimer disease suggest heritable as well as not evidently heritable forms of this disorder, consistent with genetic heterogeneity. Measurements of brain blood flow using positron emission tomography, during cognitive stimulation, suggest that reversible synaptic failure underlies early functional deficits in Alzheimer disease.

The critical temperature for maintaining stable lipid monolayers in vitro is reduced from 37 oC to less than 30 oC, using lipids from temporal association but not cerebellar cortex of Alzheimer brain. Cell membrane instability in vulnerable brain regions likely contributes to progression of Alzheimer disease.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AG 00133-11 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the <u>Clinical Pharmacokinetics, Pharmacodynamics and Therapeutics</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal PI:            T. Soncrant                      Medical Officer                      LN, NIA		
Others:       S. Asthana                      Assistant Professor                  Univ. Washington J. Kelly                              NRC Fellow                          LCMB, NIA K. Raffaele                      Scientist                              FDA N. Greig                              Visiting Scientist                  LN, NIA H. Holloway                      Biologist                              LN, NIA J. Haxby                              Staff Psychologist                  LN, NIA		
COOPERATING UNITS (if any) College of Pharmacy, University of Saskatchewan		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Cerebral Physiology and Metabolism		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 4.5	PROFESSIONAL: 4.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <u>Arecoline</u> , a <u>cholinergic agonist</u> , improves <u>memory</u> in patients with <u>Alzheimer's disease</u> when given by continuous intravenous infusion. The degree of improvement follows an inverted U-shaped relation to dose, and is maximal at a dose at least ten-fold below that producing toxicity. A <u>plasma concentration</u> was identified that achieves maximal cognitive improvement in responding patients. <u>Physostigmine</u> , a <u>cholinesterase inhibitor</u> , given by continuous intravenous infusion improved cognition in some subjects with Alzheimer's disease, but was associated with many adverse effects. Administration of <u>haloperidol</u> , a <u>dopamine antagonist</u> , produced greater cognitive and <u>motor effects</u> in young rather than in <u>aged</u> healthy men, suggesting that responsivity of the <u>brain</u> dopamine system is reduced with age in humans.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH  
SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

**TITLE OF PROJECT** (80 characters or less. Title must fit on one line between the  
Molecular Biology of Brain Aging and Disease

PI:	K.Chandrasekaran	Visiting Scientist	LN, NIA
	J.Stoll	Senior Staff Fellow	LN, NIA
	R.Fukuyama	Visiting Associate	LN, NIA

COOPERATING UNITS (if any)

LAB / BRANCH

SECTION

INSTITUTE AND LOCATION

**TOTAL STAFF YEARS:**

2.50

CHECK APPROPRIATE BOX(ES)

- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH  
SERVICE

PROJECT NUMBER

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00132-09 LN

## PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the  
Neuronal Development in Tissue Culture

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI:	Z. Galdzicki	Visiting Associate	LN, NIA
	L. Acevedo	IRTA Fellow	LN, NIA
	R. Pearce	Visiting Fellow	LN, NIA

Others:	A. Balbo	Biologist	LN, NIA
	E. Coan	Research Scientist	Univ. Birmingham
	G. Ehrenstein	Chief	

## COOPERATING UNITS (if any)

Cellular and Molecular Neuroscience Group, Medical School, University of  
Birmingham, UK; Laboratory of Biophysics, N.

## LAB/BRANCH

Laboratory of Neurosciences

## SECTION

Cerebral Physiology and Metabolism

## INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

## TOTAL STAFF YEARS:

4.0

## PROFESSIONAL:

4.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human      ☐ (b) Human      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cultured hippocampal neurons from fetal mouse trisomy 16 (Tsl6), a model for Down syndrome, showed a significant decrease in the voltage-dependent sodium current compared to diploid hippocampal neurons. High voltage-activated calcium currents, were significantly larger in trisomic neurons compared to controls. Withdrawing nerve growth factor (NGF) from the culture medium of diploid dorsal root ganglion (DRG) neurons resulted in a smaller outward potassium current compared to NGF-containing medium. Trisomy 16 DRG neurons grown in medium without NGF displayed a larger potassium current. Depolarization rate of the action potential and sodium current were unaffected by NGF in control and trisomic neurons.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH  
SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00120-16 LN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the  
Drug Development and Delivery to the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal  
PI N. H. Greig Visiting Scientist LN, NIA

Others: X. F. Pei Visiting Fellow LN, NIA  
T. Soncrant Unit Chief LN, NIA  
A. Brossi Professor Georgetown Univ.  
R. Rothman Chief LCS, NIDA  
D. Ingram Section Chief LCMB, NIA  
P. Torrence Section Chief LC, NIDDK

COOPERATING UNITS (if any)

Chemistry Dept., Georgetown Univ., LCS, NIDA; LC, NIDDK; LCMB, GRC/NIA

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  
The factors that co-determine the amount of drug that enters and is maintained in brain following its systemic administration were analyzed to aide in the safer use of drugs in the elderly and the development of central nervous system therapeutics. Rationale strategies were developed to design selective, long-duration and centrally active cholinesterase inhibitors for the palliative treatment of Alzheimer's disease and agents also were developed to improve the treatment of malignant brain tumors. Specifically, novel analogues of the alkaloid physostigmine were developed as cognitive enhancers and a novel lipophilic anticancer alkylating agents, nitrogon mustards, were developed for brain tumor treatment and treatment of tumors arising or metastasizing to breast, ovaries and lymphatics. Further, therapeutics and strategies are being developed for the treatment of neurologic diseases, of drug abuse and to improve drug delivery to brain.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 AG 00134-10 LN
<b>PERIOD COVERED</b> October 1, 1992 to September 30, 1993		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Brain Phospholipid Metabolism Relation to Function Aging and Disease)</b>		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)</b>		
PI:	D. Purdon                      Special Expert U. Shetty                      Senior Staff Fellow M. Chang                      Senior Staff Fellow C. Jones                      N.R.C. Associate W. Williams                   Guest Worker E. Grange                    Visiting Fellow N. Appel                      Guest Scientist S. Rapoport                  Lab Chief	LN, NIA LN, NIA LN, NIA LN, NIA LN, NIA LN, NIA LN, NIA LN, NIA
<b>COOPERATING UNITS (if any)</b> Dept. of Nuclear Medicine, Clinical Center NIH; Food and Drug Administration (CDER/ORR/DRT; CDER/ODE1/DNDP; CDER/ODE1/DOPOP) and Proctor and Gamble		
<b>LAB/BRANCH</b> Laboratory of Neurosciences		
<b>SECTION</b> Cerebral Physiology and Metabolism		
<b>INSTITUTE AND LOCATION</b> NIA, NIH, Bethesda, Maryland 20892		
<b>TOTAL STAFF YEARS:</b> 5.0	<b>PROFESSIONAL:</b> 5.0	<b>OTHER:</b> 0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b> <p>           A <u>mathematical model</u> was used to derive <u>operational equations</u> for examining <u>incorporation and turnover of fatty acids</u> within individual <u>brain phospholipids</u>, under in vivo conditions in animals and humans. In rats, the model was combined with <u>quantitative autoradiography</u> and chemical analysis to examine these parameters with the saturated [9,10-3H]<u>palmitic acid</u> (3H-PA) and the unsaturated [14C]<u>arachidonic</u> (14C-AA) and [14C]<u>docosahexaenoic acids</u> (14C-DHA). Administration of <u>serotonergic agonists</u> indicated involvement of 14C-AA but not 3H-PA in <u>second messenger systems</u>. <u>Pentobarbital</u> reduced incorporation of 3H-PA into brain, consistent with its GABAergic effect or direct enzymatic action on palmitate turnover. Rates of incorporation of the three labels were increased 3-5 fold compared to normal brain by cerebrally implanted <u>Walker 256 carcinomasarcoma cells</u> in rats, indicating that the method can be used to <u>image brain tumors</u> in vivo. Differential uptake of the three labels into different phospholipids suggested tumor specificity. In situ perfusion and fractionation of brain membranes and of precursor pools indicated very rapid equilibration of specific activity or radiolabeled plasma fatty acids with <u>precursor pools</u>, but that most of the contribution to the precursor pools comes from de novo synthesis and/or recycling from brain lipids. <u>Age</u> related changes in mouse brain <u>capillary</u> composition suggested a role for <u>free radical oxidation</u>. An inhibitor of <u>fatty acid oxidation</u>, increased the fraction of labeled palmitate that entered brain lipids and was not oxidized, suggesting its use in <u>positron emission tomography (PET)</u>. [11C]-labeled positron emitting fatty acids were synthesized and shown to give incorporation coefficients in <u>monkeys</u> comparable to those in <u>rats</u>, with in vivo imaging by PET, suggesting use for studying clinical brain disease. Rates of fatty acid incorporation were shown by theory and experiment to be independent of <u>cerebral blood flow</u>.         </p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AG 00128-13 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the <u>Analytical Drug Methods</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal PI: U. Shetty Sr. Staff Fellow LN, NIA		
OTHERS: H. Holloway Biologist LN, NIA		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Cerebral Physiology and Metabolism		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland, 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input checked="" type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A highly reproducible <u>mass spectrometric assay</u> for <u>myo-inositol</u> in <u>cerebrospinal fluid</u> and <u>plasma</u> was developed. Assay development for other <u>polyols</u> by a similar technique is in progress.  An analytical method using <u>high performance liquid chromatography</u> (HPLC) with fluorescence detection was developed for the measurement of <u>physostigmine</u> , a <u>cholinesterase inhibitor</u> , in <u>plasma</u> of humans receiving the drug as treatment for Alzheimer's disease. A <u>gas chromatographic/mass spectrometric assay</u> for the measurement of <u>nicotine</u> in rat plasma and brain, and in human plasma was developed. Highly reproducible mass spectrometric assay for myo-inositol in cerebrospinal fluid and plasma was also developed. Assay development for other polyols by a similar technique is in progress.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH  
SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00125-14 LN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Cerebral Metabolism, Relation to Brain Function and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI: T. Soncrant Medical Officer LN, NIA  
E. De Micheli Visiting Fellow LN, NIA

Others: H. Holloway Biologist LN, NIA  
D. Larson Biologist LN, NIA  
N. Greig Visiting Scientist LN, NIA  
U. Freo Visiting Fellow LN, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

5.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Changes in regional cerebral metabolic rates for glucose produced by the serotonin agonist m-chlorophenylpiperazine (m-CPP) are reduced by chronic pretreatment with m-CPP in both young and old rats, demonstrating preservation of serotonin regulation during aging. The metabolic effects of m-CPP depend upon adrenal function. In a model of cholinergic cortical deafferentation, produced by lesioning the nucleus basalis magnocellularis cerebral metabolic deficits were smaller in aged rats, demonstrating an age-related reduction in tonic cholinergic input to the neocortex. Metabolic responses to cholinergic drugs were preserved in this model, indicating presynaptic reorganization also manifest as altered regional brain functional interactions.

This project has been terminated.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER 201 AG 00123-15 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Neuronal Development in Tissue Culture		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal		
PI:	Z. Galdzicki	Visiting Associate LN, NIA
	L. Acevedo	IRTA Fellow LN, NIA
	R. Pearce	Visiting Fellow LN, NIA
Others:	A. Balbo	Biologist LN, NIA
	E. Coan	Research Scientist Univ. Birmingham
	G. Ehrenstein	Chief
COOPERATING UNITS (if any) Cellular and Molecular Neuroscience Group, Medical School, University of Birmingham, UK; Laboratory of Biophysics, N.		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Cerebral Physiology and Metabolism		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 4.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Cultured hippocampal neurons from fetal mouse trisomy 16 (Tsl6), a model for Down syndrome, showed a significant decrease in the voltage-dependent sodium current compared to diploid hippocampal neurons. High voltage-activated calcium currents, were significantly larger in trisomic neurons compared to controls. Withdrawing nerve growth factor (NGF) from the culture medium of diploid dorsal root ganglion (DRG) neurons resulted in a smaller outward potassium current compared to NGF-containing medium. Trisomy 16 DRG neurons grown in medium without NGF displayed a larger potassium current. Depolarization rate of the action potential and sodium current were unaffected by NGF in control and trisomic neurons.</u>		





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00133-10 LN

## PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the  
Clinical Pharmacokinetics, Pharmacodynamics and Therapeutics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI: T. Soncrant Medical Officer LN, NIA

Others: S. Asthana Assistant Professor Univ. Washington  
J. Kelly NRC Fellow LCMB, NIA  
K. Raffaele Scientist FDA  
N. Greig Visiting Scientist LN, NIA  
H. Holloway Biologist LN, NIA  
J. Haxby Staff Psychologist LN, NIA

## COOPERATING UNITS (if any)

College of Pharmacy, University of Saskatchewan

## LAB/BRANCH

Laboratory of Neurosciences

## SECTION

Cerebral Physiology and Metabolism

## INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.5

OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human ☐ (b) Human ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

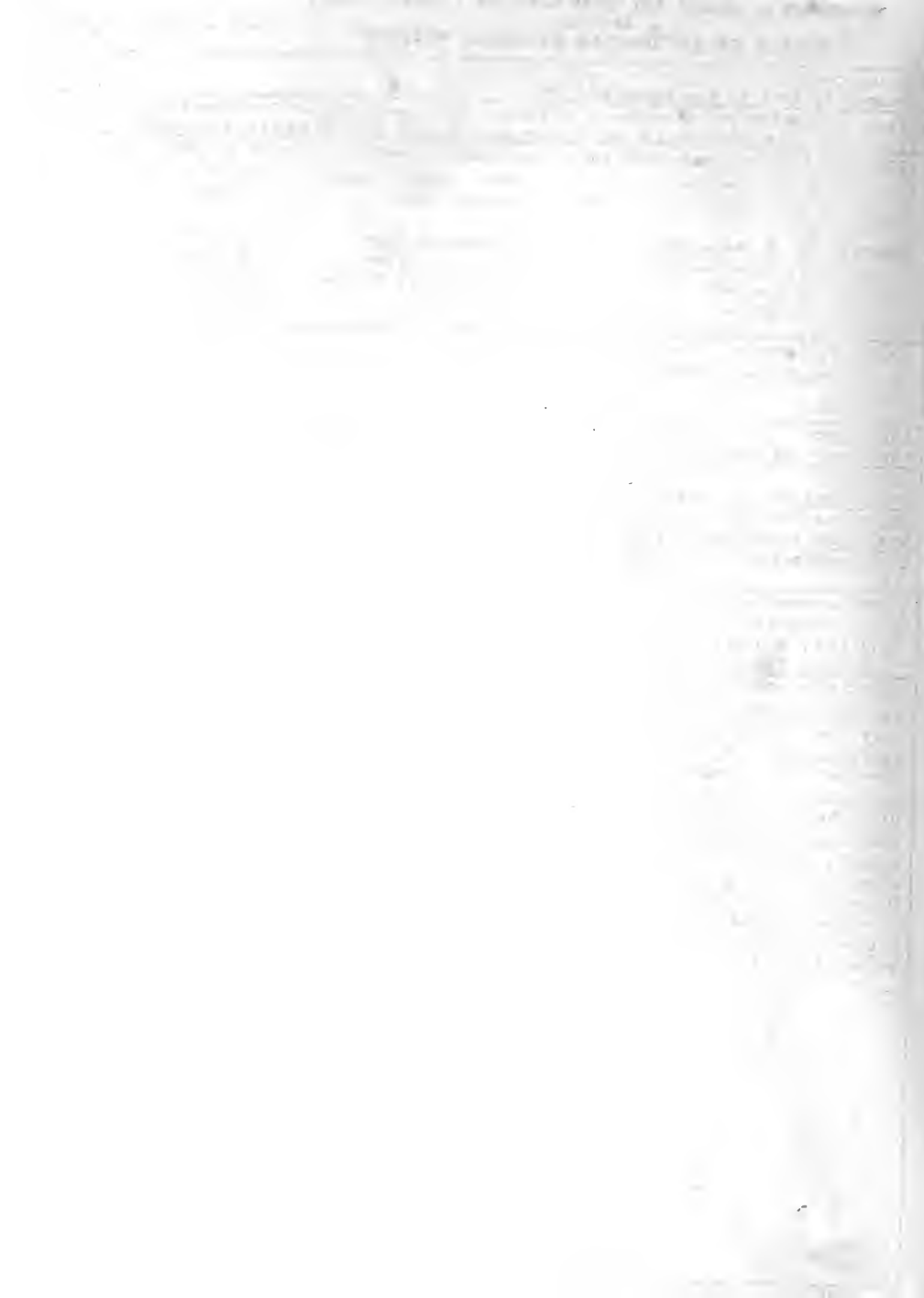
Arecoline, a cholinergic agonist, improves memory in patients with Alzheimer's disease when given by continuous intravenous infusion. The degree of improvement follows an inverted U-shaped relation to dose, and is maximal at a dose at least ten-fold below that producing toxicity. A plasma concentration was identified that achieves maximal cognitive improvement in responding patients. Physostigmine, a cholinesterase inhibitor, given by continuous intravenous infusion improved cognition in some subjects with Alzheimer's disease, but was associated with many adverse effects. Administration of haloperidol, a dopamine antagonist, produced greater cognitive and motor effects in young rather than in aged healthy men, suggesting that responsivity of the brain dopamine system is reduced with age in humans.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AG 00129-13 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the <u>Blood-Brain Barrier and the Regulation of Brain Nutrient and Metal</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal PI:      Q. R. Smith                      Chief, SNBT                      LN, NIA K. C. Wadhvani                  Senior Staff Fellow            LN, NIA Q. Deng                              Visiting Associate            LN, NIA Y. Kohmo                            Visiting Fellow                LN, NIA  Others: S. I. Rapoport                  Chief, LN                          LN, NIA J. Stoll                                Staff Fellow                      LN, NIA O. Rabin                               Guest Worker                    INSERM, Paris, France		
COOPERATING UNITS (if any) Unit on Neurotoxicology, INSERM, Paris, France; National Institute of Standards and Technology; Biomedical Engineering and Instrumentation Branch, NIH; Frederick Cancer Research Center, NCI, Frederick, MD.		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Neurochemistry and Brain Transport		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>A highly sensitive secondary ion mass spectrometry</u> method was developed to image distributions of trace elements in frozen sections of postmortem human brain. Studies were initiated on the distribution of <u>aluminum</u> and other key elements in <u>neurofibrillary tangle-bearing neurons</u> in Alzheimer's disease. The brain transport of manganese, an essential trace metal that produces a Parkinsonian-like syndrome at high concentrations, was studied in rats and found to be mediated by an uptake system that is influenced by plasma protein binding, oxidation state and trace metal competition. The <u>basic amino acid transporter</u> at the <u>blood-brain</u> <u>barrier</u> was cloned and shown to be homologous to System y <sup>+</sup> , the classic sodium-independent cationic amino acid carrier. Drugs with high affinity for the <u>large neutral amino acid transporter</u> of the blood-brain barrier were identified and tested for rapid uptake into brain.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 AG 00121-16 LN
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the <u>Function and Structure of Blood-Nerve and Blood-Brain Barriers</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal		
PI:	K. C. Wadhwani	Senior Staff Fellow LN, NIA
	Q. R. Smith	Section Chief LN, NIA
Others:	S. I. Rapoport	Laboratory Chief LN, NIA
	J. Koistinaho	Visiting Fellow LN, NIA
	C. Latker	Senior Staff Fellow LN, NIA
	A. Balbo	Technician LN, NIA
	V. Murphy	Senior Staff Fellow LN, NIA
COOPERATING UNITS (if any) US Uniformed Health Services, MD		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Neurochemistry and Brain Transport		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Blood-brain and blood-nerve barrier permeabilities to ions and nonelectrolytes</u> are very <u>low</u> , indicating limited exchange between plasma and brain or nerve <u>extracellular compartments</u> . The nerve barrier, unlike the <u>blood-brain barrier</u> , does not have a regulatory transport system for <u>calcium</u> . In the <u>rat</u> , the <u>integrity of both barriers</u> to small nonelectrolytes is <u>maintained with age</u> . The blood-brain and blood-nerve barriers have <u>regulated carriers</u> for the transfer of <u>manganese, neutral amino acids, and basic amino acids</u> . In addition, <u>cationized albumin</u> is taken up into nerve at a greater rate than native albumin, possibly by receptor-mediated transcytosis. Similar properties have been observed at the blood-brain barrier. A modified <u>polymerase chain reaction titration method</u> was developed to quantify <u>glucose transporter mRNA</u> expression at the cerebral microvessels. Various techniques were developed to modify blood-brain barrier permeability and transport for <u>chemotherapy</u> of brain tumors.		



EDBP





ANNUAL REPORT  
OF THE  
EPIDEMIOLOGY, DEMOGRAPHY, AND BIOMETRY PROGRAM  
NATIONAL INSTITUTE ON AGING

Overview

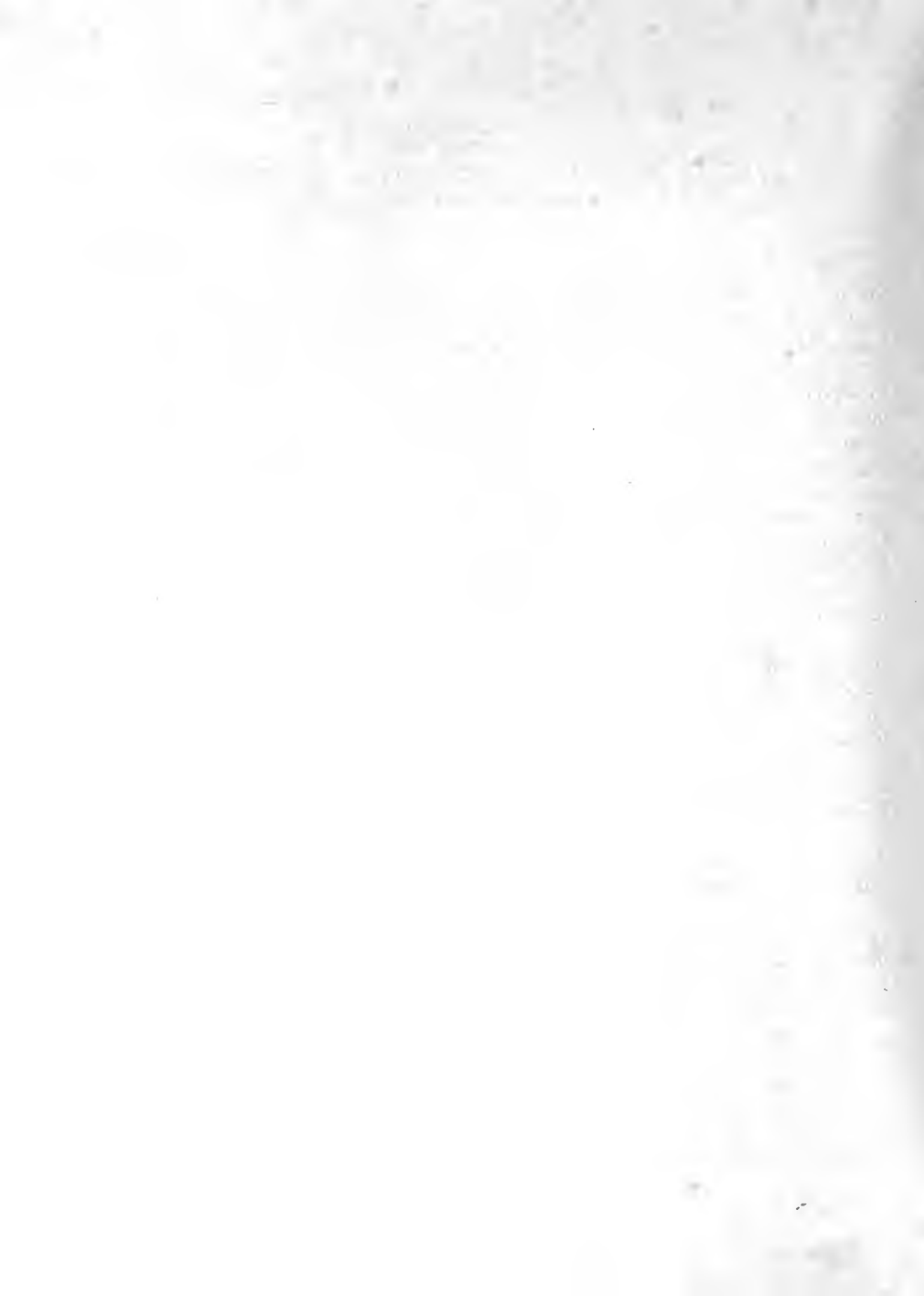
**Mission:** The Epidemiology, Demography, and Biometry Program (EDBP) (1) plans, conducts, and directs epidemiology, demography, and biometry programs relevant to the mission of the NIA; (2) collects and analyzes data regarding the distribution of the aged by such categories as sex, race, socioeconomic and demographic characteristics and serves as a focal point for these data; (3) plans, initiates, coordinates, and analyzes national and international epidemiologic longitudinal studies and studies of special populations; (4) in collaboration with NIA staff and that of other Institutes, federal agencies and scientific organizations, recommends priorities and develops epidemiologic studies of specific diseases and conditions affecting the aged; (5) provides consultation and service to NIH program areas and private organizations on epidemiology, demography, and biometry studies on aging; (6) recommends mechanisms to be used or develops mechanisms to accomplish Program objectives; (7) plans and directs research and training in the areas of epidemiology, demography, and biometry, and serves as the primary federal source of information regarding research and training in these areas.

**Introduction:** The EDBP, a component of the NIA intramural research program, based in Bethesda, Maryland, functions similarly to other NIH intramural programs. EDBP investigators identify hypotheses, initiate research studies, and report results in the scientific literature. EDBP "laboratories" are communities and populations often supported through competitive contracts to academic and community institutions. The Program has four units: the Epidemiology and Demography Office, the Asia-Pacific Office, the Geriatric Epidemiology Office, and the Biometry Office.

Activities consistent with the above mission statement are proceeding in a number of areas and are summarized below and in the individual Office reports.

**Productivity:** Highlights of published or in-press reports by EDBP staff are included with the Office reports but selected findings, most from the Established Populations for Epidemiologic Studies of the Elderly (EPESE) project, which involves longitudinal follow-up of communities, are summarized:

- The occurrence in the EPESE project of loss of mobility was predicted by various risk factors and diseases. A new heart attack, stroke, cancer or hip fracture was associated with substantially greater risk than was associated with the presence of these conditions at baseline, as was current smoking, not consuming alcohol, high body mass index, and low physical activity.



- At the age of 65, those with 12 or more years of education had an active life expectancy that was 2.4 to 3.9 years longer than those with less education, and with adjustment for education, active life expectancy was similar between blacks and whites.
- After adjustment for related factors, increased risk of pneumonia mortality was found for those with limitations in activities of daily living and cognitive impairment in both men and women.
- Septicemia mortality was associated with age, male gender, history of diabetes, smoking, disability in activities of daily living and cognitive impairment.
- A high level of recreational physical activity in the physically capable subset of the EPESE reduced the likelihood of mortality after both 3 and 6 years.
- Low education and occupation are significant predictors of development of cognitive impairment in the EPESE.
- Using data from follow-up of a national sample of white women aged 65-74 years, those with a high maximal lifetime body mass index experienced an increased risk of coronary heart disease. The relationship was enhanced when recent weight loss was accounted for in the analysis. In related analysis, lean women with stable weight had the lowest risk of all-cause mortality, while those who had lost weight have a high risk irrespective of weight.

**Study Development:** When the Program began approximately 15 years ago, descriptive data on aging and age-related diseases were lacking or concentrated in studies of a single disease, e.g., coronary heart disease or diabetes. Three community studies (EPESE) were created to fill the information gap, and they have provided much new and important data on health status in older persons. For example, a new estimate of Alzheimer's disease frequency was reported from the East Boston, Massachusetts population.

More recently, as a result of careful review and planning, data collection in these first-generation studies is now being completed; and direct contract support is being phased out. However, a study at one of the sites, focusing on black-white differences among elderly living in the Piedmont area of North Carolina will continue for 4 years. These studies have provided much useful data, which are yielding a great many valuable reports that are now being published by EDBP staff and collaborating scientists.

Based on hypotheses and data needs that cannot be addressed by these studies, a second-generation of studies has been developed. These were built on the expertise and research interests of EDBP staff and with outside consultation. Specifically, three new studies are completing an initial data collection phase. They are: the Women's Health and Aging Study, which is a study with a strong biomedical emphasis of the causes and course of disability in older community-dwelling women; the Honolulu Dementia and Aging study, an



addition to a long-term study of Japanese-American men in the Honolulu Heart Program that emphasizes potential risk factors for Alzheimer's disease and vascular dementia; and the Comorbidity and Cancer in the Elderly study, a collaboration with the National Cancer Institute and its large cancer registry program. Also, there has been an add-on of immunologic measures to the ongoing study in the biracial EPESE community in North Carolina and the addition of metabolic, immunologic factors, and body composition studies to the long-term heart disease study in Framingham, Massachusetts. Finally, review is proceeding on proposals resulting from an RFP for a study of the potential effect of head trauma and unconsciousness during the Second World War on the onset of Alzheimer's disease.

**Proposed Studies:** Efforts have been made by the EDBP leadership to incorporate biomedical issues into current and potential future EDBP research studies, and in particular to emphasize the interaction of multiple diseases (comorbidity) and the aging processes in the development of study proposals for third-generation investigations. For example, outside peer review for concept clearance and internal Contract's Advisory Committee review with recommendation to the NIA Director have been obtained for three new proposed studies:

- To enhance studies in long-lived population participating in the Honolulu Dementia and Aging Study by biomarker assessment of Apo-E status; by magnetic resonance imaging of the brain for all individuals who are either incident cases of dementia or who have an Apo-E 4 phenotype; by measuring body composition and assessing dietary habits on those long-lived persons who have different patterns of lifelong weight change.
- The Health ABC, a study of early decline in function related to change in body composition and decline in health status, targeting disease and metabolic relationships in healthier older whites and African-Americans. A vitality component may be added.
- To study the influence of age on the choice of cancer treatment made by physicians for their elderly patients. Information is being collected on cancer and comorbidity, and there are already plans to obtain data on illness behavior and quality of life from cancer patients.

**Staff Development and Training Functions:** Over the years the core staff have been built up through recruitment of productive trainees and external subject matter specialists. Trainees through the NIH Intramural Research Training Award Program and the USPHS Epidemiology Training Program have worked in EDBP. The latter program usually involves 1 year at a school of public health and 2 years with the Program. Currently two individuals Dr. Lori Brown, formerly of a Stanford University immunology lab and Dr. John Sorkin, a Senior Staff Fellow at the GRC, are in this epidemiology training program. Also, the rich experience and talents of foreign scientists have been utilized by the Program. Drs. Marco Pahor and Antonio Sgadari from Rome spent 3 months with the Program, and Dr. Chiara Corti has joined us for a year or more as a Visiting Fellow after one year of training in epidemiology at Johns Hopkins.



We have recruited Dr. Jean Langlois, an experienced injury studies investigator, as well as a general epidemiologist with background in speech pathology. Efforts are proceeding to fill a long vacant biostatistician position. A well trained statistician will help immensely in developing more appropriate statistical methods and sustaining the in-house capacity necessary for supporting active epidemiologic research.

**Consulting and Interagency Cooperation:** The intramural program has the potential for interrelationships with other institutes, government agencies, and the extramural community. A number of these collegial arrangements are detailed in the Office reports. As a representative example, the addition of a dementia component to the Honolulu Heart Program is being accomplished through an intraagency agreement with the originating institute. However, the impact of the study is being potentiated by intramural collaboration with other governmental and extramural groups. Comparison with Japanese studies is made possible through long-term Department of Energy sponsored studies. Grant supported research, including an administrative supplement, has allowed cross-national comparisons with Seattle, Washington. Workshops on various topics, such as the use of objective performance measures of functioning, immunologic indicators of aging, and cancer in the elderly have been convened as another way to promote interchange with the community.

**Epidemiologic Directions:** There is a continuing process of development and reappraisal of research directions of the Program by staff and outside groups. Previously, an ad hoc scientific advisory committee met regularly and provided oversight for the Program. As a result of their advice, there has been a definite recent emphasis on the identification, where possible, of biologic mechanisms as they relate to key aging issues being addressed by ongoing and planned epidemiologic studies. A complementarity of purpose exists between the EDBP and the larger NIA Intramural Research Program (IRP). The rationale for such linkage is consistent with observations made by Dr. Lilienfeld, a major contributor to the development of modern epidemiology. He has listed three general ways that information from epidemiologic studies can be used: "(1) To elucidate the etiology of a specific disease or group of diseases by combining epidemiologic data with information from other disciplines such as genetics, biochemistry and microbiology; (2) To evaluate the consistency of epidemiologic data with etiologic hypotheses developed either clinically (at the bedside) or experimentally (in the laboratory) (3) To provide the basis for developing and evaluating preventive procedures and public health practices." It is the public health potential that requires large and sometimes costly community and population-based studies dealing with aging and age-related diseases and that differentiates EDBP somewhat from the IRP. The goal of such studies is to generate sufficient population-based knowledge to make changes that would move the entire population of older persons to a more advantageous and beneficial location on the risk spectrum. These are the types of studies that EDBP staff need to continue developing, completing, and reporting on now and in the future.





### Specific Activities of the Associate Director

Dr. Richard Havlik, Associate Director, EDBP is a member and Chairperson of the PHS Epidemiology Training Program Steering Committee; a member of the NIH Epidemiology Coordinating Committee, and the Biomedical Scientific Research Service Credentialing Committee. Dr. Havlik is the NIH representative to the National Death Index Advisory Committee. He gave presentations on "Comorbidity and Disability in Older-Aged Persons" to the Trieste Workshop on Cancer in the Elderly; and "Epidemiology of Hypertension: Relationship to Glucose Intolerance" to a Consensus Development Conference on Treatment of Hypertension in Diabetes organized by the American Diabetes Association. He will present in September 1993 at the annual meeting of the International Association of Cancer Registries meeting in Bratislava, Slovak Republic. He collaborated with NIA staff members on a chapter entitled "Epidemiology and Treatment of Hypertension in Older Persons" for Hypertension: Pathophysiology, Diagnosis and Management; "Epidemiology and Demography" for the Merck Manual of Geriatrics; and an editorial on "Epidemiology of Aging" for the Annals of Epidemiology.

### Cancer in the Aged Subprogram

The NIA Epidemiology, Demography, and Biometry (EDB) Program began several research projects to address the cancer/aging interface in 1991. We conduct these studies in collaboration with colleagues in the National Cancer Institute (NCI).

Cancer is a leading cause of morbidity and mortality in the United States for all age groups, but it is a special problem for persons 65 years and older. Over 55 percent of all cancers occur in this subset of the population; 67 percent of cancer mortality is in this age group. In the U.S. the major tumors affecting older-aged persons are breast, prostate, colon, rectum, lung, urinary bladder, pancreas, and stomach. Only recently have these facts become recognized. Therefore, the management of cancer in older-aged patients has not been studied to any large extent. Since older cancer patients have health care needs independent of and associated with their cancer diagnosis, EDB projects address the information gaps on cancer in the elderly, especially those concerning comorbid conditions present at initial diagnosis of cancer, multiple tumors, illness behavior, and quality of life issues in surviving cancer.

EDB and the NCI Surveillance, Epidemiology, and End Results (SEER) Program have instigated development of cross-national projects that utilize the strength of selected tumor registries in Italy, the Netherlands, and the Slovak Republic to address several of these topics. Comparative international studies can provide many useful answers to questions of etiology, early detection, treatment, and the influence of aging on cancer patient management.



## NIA/NCI COLLABORATIVE STUDY: COMORBIDITY AND EARLY DIAGNOSIS OF CANCER IN THE ELDERLY

The NIA and the NCI are conducting a study on cancer in the elderly using resources of the NIA EDB Program and the NCI SEER Program. Seven cancers have been designated for the NIA/NCI SEER Collaborative Study -- colon, stomach, urinary bladder, prostate, breast, ovary, and cervix uteri tumors. Study objectives are to:

- **Develop descriptive information** in response to the basic questions of what are the predominant comorbid conditions and functional limitations that exist in older-aged persons diagnosed with cancer; and what do older-aged persons do when they become aware of themselves as having cancer-suspicious signs and symptoms.
- **Develop a profile of comorbidity** and ascertain the extent to which competing health problems influence treatment of older persons diagnosed with cancer.
- **Assess illness behavior** in the elderly as a precursor to taking action for cancer signs and symptoms.
- **Generate information for application of research** into practice, promote studies on clinical interventions, and hypothesis testing to more effectively address the unique problems of cancer in aged persons (i.e., minimize morbidity, prevent loss of function, promote quality of life in parallel with appropriate medical and surgical treatment).

The major questions are: (1) do older-aged persons receive the same type of initial treatment procedures as younger persons for the same cancer sites (controlling for disease stage); (2) to what extent are concomitant disease and/or illnesses and the normal processes of aging associated with cancer treatment in older-aged persons; and (3) are older-aged persons more likely to present with advanced stages of cancer at initial diagnosis; and (4) what factors increase the length of time from recognition of symptoms to the time of diagnosis so that they are more likely to be seen in later stage disease.

The NIA/NCI SEER Collaborative Study is an "add-on" to the routine operation of six of the nine SEER Program participants -- the states of Iowa, Utah, and New Mexico, and the reporting regions of Seattle, Washington, San Francisco-Oakland, California, and Atlanta, Georgia. SEER cancer registry participants in designated U.S. geographic areas abstract information on incident cancers from hospitals in their designated regions. The large scale population-based SEER data system covers about 10 percent of the U.S. population and includes the states of Connecticut, Hawaii, and Detroit, Michigan in addition to those mentioned above. Principal investigators are responsible for consolidating all data concerning an individual cancer case and assuring quality control procedures.



The study's pilot phase (on 1712 patient records) was completed in November 1992. The pilot study established study procedures, verified efficacy of data collection forms, and created the study infrastructure to obtain information on comorbidity from medical records of cancer patients. Other information derived from the pilot study on comorbidities present, disease symptomatology, and medications at admission to the hospital provided the foundation for the full field operation (April 1993). Data will be obtained on a random sample of 7800 cancer patients with an age emphasis on those 65 years and older. A control group of patients aged 55-64 years is included. The field operation, Phase II, is scheduled for April 1, 1993 through March 31, 1994.

Instruments for the illness behavior pilot study, Phase III, will be pre-tested in late summer, early fall, 1993. The interview component of the NIA/NCI SEER Collaborative Study is scheduled to begin in early 1994; however, additional funding for this phase will need to be identified.

The NIA project officers for the NIA/NCI SEER Collaborative Study are Drs. Rosemary Yancik and Richard Havlik. NCI project officer is Dr. Brenda Edwards, Surveillance Program, Division of Cancer Prevention and Control.

## **IOWA EPESE/SEER STUDY ON CANCER IN THE ELDERLY**

The cancer experience of 737 Iowa EPESE respondents is being investigated by Drs. Rosemary Yancik and Richard Havlik, other EDBP staff, and Dr. Jon Lemke, University of Iowa. The Iowa data from the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Program, a population-based national tumor registry were merged with data from the Iowa EPESE study participants. Development of the Iowa EPESE/SEER database was completed in 1992. The SEER data augment the subgroup of EPESE participants diagnosed with cancer providing the following information: (1) extent of disease at initial diagnosis (e.g., stage of the malignancy); (2) natural history of the tumor; (3) initial treatment; and (4) details on survival over time. Information is available to respond to the following questions:

### **Characterization of the IOWA/SEER cancer survivor cohort**

1. What was the extent of each malignancy for the EPESE cancer patient/survivor at initial diagnosis (i.e., disease stage) according to specific cancer site in the baseline group?
2. What is the extent of the malignancy for the EPESE cancer patient/survivor group who developed cancer subsequent to the baseline interview? What predisposing factors can be distinguished for this group (e.g., symptoms)?
3. What chronic conditions are likely to be found in older persons with cancer? Were these present at initial diagnosis of the tumor?



4. On what social and health behavioral dimensions do persons with cancer and multiple comorbid conditions differ from those who have cancer as a single condition?

#### **Cancer patient/survivors compared with those afflicted with other chronic diseases**

5. What is the self-perceived health status of the cancer only patient/survivor group as compared with cancer and other chronic conditions patient/survivor group? How does this compare with the self-perceived health status of those not afflicted, controlling for cancer site and age?
6. Do the number and severity of the chronic conditions associated with cancer differ for those EPESE participants with heart disease?
7. Does the functional status of EPESE participants who have had a diagnosis of cancer differ from other participants diagnosed with other chronic diseases or those without a chronic disease.
8. What coping mechanisms or resources are used by cancer patients/survivors as compared with patients/survivors of other chronic diseases?
9. What deleterious behavioral factors (e.g., smoking) are associated with the complex of comorbidity and cancer.
10. What is the impact of cancer on the social functioning of older-aged persons as compared with those who have not been afflicted?

#### **FOLLOW-UP: NIA/NCI/FOGARTY CENTER CENTRAL AND EAST EUROPEAN INITIATIVE**

In 1991, with support from the National Institutes of Health (NIH) Fogarty International Center (FIC) Central and Eastern European Initiative (CEEI) and their respective Institutes, an NIA/NCI study team examined the potential for conducting comparative international studies on cancer in the aged with five registries -- two in Italy (in Trieste and Milan); one in the Slovak Republic (Bratislava); and two in Poland (in Krakow and Warsaw). In a separate exchange, the Eindhoven Tumor Registry, the Netherlands, was also visited. The focus was on the data available from these population-based tumor registries to compare with the population-based data collected by the NCI SEER Program regarding the common concerns on cancer in persons aged 65 years and older.

Our purpose was to foster scientific collaboration in the areas of mutual interest on aging and cancer relevant to geriatrics, gerontology, and oncology and to establish a regular exchange of scientific information and encourage exchange of scientists between the U.S. and each of the countries. The NIA and NCI Study Team discussed the scope and feasibility of





international collaboration on a country-by-country and registry-by-registry basis. It was not intended to develop a multi-national comparative study. Differences and similarities in the incidence and mortality rates of cancers between the U.S. and the selected nations offer insights on the high risk of acquiring cancer associated with advancing age, a phenomenon observed in the U.S. consistent with the data in Italy, Slovakia, and Poland. Our commitment to expand the knowledge base on cancer in the aged in both Institutes via appropriate tumor registries capable of cross-national research was implemented in 1992. Collaborative activities to date are:

- In December 1992, Dr. Giorgio Stanta, director of the Trieste Tumor Registry, with support from the local government, organized a scientific conference on cancer in the elderly.
- The Bratislava, Slovak Republic, Trieste, Italy, and Eindhoven, Netherlands Tumor Registries are conducting pilot studies using the NIA/NCI comorbidity form to access the medical records of older-aged patients to ascertain the logistics and feasibility of designing a prospective study similar to the NIA/NCI study.
- The Trieste Tumor Registry is characterizing the comorbidity associated with cancer in older patients revealed at autopsy and designing a study to look at the natural history of the tumor, biopsy to necropsy.
- The Trieste centenarian study is being expanded with respect to tracing the cancer burden and extending the data base to include nonagenarians (i.e., "Health and Coping Experiences in the Tenth Decade of Life").
- Studies of the prominence of multiple tumors in the elderly with the tumor registry data are underway. Multiplicity of tumors is more common in older-aged cancer patients because of the long period of development of subsequent tumors. The Trieste data, based as it is on an expansive pathology of the deceased cancer patient, has an optimum opportunity to secure this type of information.

#### CANCER IN AN AGING WORLD: INTERCOUNTRY VARIATIONS IN INCIDENCE, MORTALITY, AND SURVIVAL OF PERSONS 65 YEARS AND OLDER

The NIA and the NCI proposed to the International Association of Cancer Registries (IACR) colleagues that an international symposium on cancer and aging be organized. Certainly, the ideas expressed above concerning patients 65 years and older may be extended to other countries for cross-national research. The NIA Office of International Activities issued a professional services contract with IACR in August 1992, to organize and support the cancer in an aging world workshop as an adjunct to the IACR annual meeting. The current membership of IACR is about 240 cancer registries (Africa 12, Asia 33, Oceania 15, Europe, 105, North America 23, and South America 20). The IACR symposium will occur



at the September 1993 annual meeting in Bratislava, Slovak Republic. Selected papers will be published as a proceedings.

The issues to be addressed by participants in the annual meeting include:

1. What are the differences in the cancer profiles according to age for the major malignancies in a particular nation?
2. Are older-aged persons more likely to present with advanced stages of cancer at initial diagnosis?
3. Do older-aged persons receive the same type of surgical treatment procedures as younger persons?
4. What are the differences in relative survival experience of older-aged persons as compared to younger-aged persons?
5. Are data available to demonstrate associated comorbid conditions and functional status in older-aged cancer patients?

Participants in these efforts are NIA staff, Drs. Rosemary Yancik and Richard Havlik and NCI staff, Dr. Brenda K. Edwards and Ms. Lynn A. Ries.



## **Research Highlights FY93**

- The EPESE project has developed information on death, chronic conditions, disabilities, and institutionalization for representative samples of elderly people living in communities. The EPESE consists of prospective epidemiologic studies of approximately 14,000 persons 65 years of age and older in 4 different communities: East Boston, Massachusetts; two rural counties in Iowa; New Haven, Connecticut; and segments of 5 counties in the north-central Piedmont area of North Carolina. The study design includes an initial baseline household interview followed by continued surveillance of morbidity and mortality. Participants were re-contacted annually in conjunction with the collection of data on cause of death and factors related to hospitalization and nursing home admissions. Concurrently, the investigators developed substudies focused on specific problems of the elderly. The value of this research lies in the longitudinal design which allows for analyses aimed at identifying risk factors of diseases, disabilities, hospitalizations, institutionalization, and mortality. (Comoni-Huntley, Ostfeld, Taylor, Wallace, Blazer, Berkman, Evans, Kohout, Lemke, Scherr, Korper. *Aging Clin Exp Res*, 1993;5:27-37.)
- Age comparisons for incidence, histology, disease stage at initial diagnosis, and mortality of more than 20,000 ovarian cancer patients diagnosed between 1973-1987 were the focus of this descriptive epidemiologic study. Key issues and concerns were highlighted regarding ovarian cancer in women 65 years and older as a frame of reference for the proceedings of the working conference, "Perspectives on Ovarian Cancer in Older-Aged Women," co-sponsored by NIA, NCI, and the American Cancer Society held at the NIH, November 1991. Data were from the NCI's Surveillance, Epidemiology, and End Results (SEER) Program and the National Center for Health Statistics. The SEER Program database represents approximately 9.6% of the U.S. population. Ovarian cancer affects women in the age group 65 years and older more frequently than younger women. More than 48% of all ovarian cancers occur in women in this age group. Age-adjusted rates increase as age advances, peaking at 54.0 per 100,000 in the age group 75-79 years. Time trends also indicate increases in age-specific incidence rates. This malignancy takes its toll in mortality in women 65 years and older with 64% of all deaths due to this neoplasm (in 1989). Moreover, older women are more likely to be initially diagnosed with advanced disease. Important questions about ovarian cancer in older-aged women need urgent attention from the research community. New strategies for diagnostic leads have to be developed for older women. (Yancik. *Cancer*, 1993;71:517-23.)



## CONTRACT

Name and Number: The Johns Hopkins University School of Medicine (N01-AG-1-2112)

Title: Women's Health and Aging Study

Date Contract Initiated: July 1, 1991

Current Annual Level: \$1,877,654

**Objectives:** The overall goal of the study is to understand the causes and course of physical disability (defined as a deviation or alteration in normal functional performance) in older women living in the community. This will be accomplished by (1) screening a representative population of community-dwelling older women to recruit a study cohort of women with moderate to severe dependence in physical functioning, (2) characterizing prevalent diseases and conditions in members of this cohort and assessing the impact of these conditions on physical function, and (3) following the cohort prospectively for a period of 3 years to evaluate change in functional status.

**Methods Employed:** Participants in the study are selected at random from among women aged 65 and over living in community residences in 12 zip code areas of Baltimore City and County. The initial sample will include 5,500 women, stratified by age so as to ensure sufficient numbers in the oldest age groups. A brief screening interview will identify approximately 1,270 women who are moderately to severely disabled, defined as being disabled in 2 or more of 4 specified domains of functioning. Of these, about 1,000 are expected to agree to participate in the remainder of the study. These women will complete a baseline interview and an examination conducted in their homes by a nurse practitioner that includes performance-based measures of disability. Follow-up interviews will be conducted with participants every 6 months for the next 3 years.

**Significance to Biomedical Research:** This study has a number of aspects that make it unique as an epidemiologic study and add substantially to previous work done on disability in older populations. Epidemiologic studies in general have tended to study factors leading to the onset of incident disease, with little study of subjects once disease occurs. This study will examine women who already have disease and disability, attempt to understand the diseases underlying that disability and then prospectively evaluate the course of disability and how the underlying diseases as well as health habits, psychological, cognitive, social and other factors affect that course. Unlike previous epidemiologic research on disability, this study will intensively evaluate diseases and physiologic dysfunction that are associated with disability.

**Proposed course:** The first 15 months of the study were primarily concerned with planning, development of assessment protocols, creation of an OMB package, and pre-testing of questionnaire and examination instruments. Fieldwork began in November 1992. Baseline assessments, which include an interview, an examination by a nurse, and obtaining a blood





sample, will run through July 1994. Follow-up assessments, which will be done every 6 months for 3 years, began in May. Reports from field supervisors, interviewers, and nurses indicate that the study is being well received by participants, no serious difficulties have been encountered, and screening and recruitment of eligible women into the study has gone well. The use of Computer Assisted Personal Interviewing (CAPI) is a major benefit to the data collection activity, allowing for analysis of data soon after the interview is completed.

The sampling for this study has been designed to stratify the full target population into four replicates, each representative of the entire geographic area. As of July 20, 1993, 823 women from the first replicate have been screened, and 296 (36.0%) have been found to be eligible for the study. Included in these numbers are participants in the "First Fifty Study," composed of a stratified random subsample of 309 women in the first replicate. This cohort of women was created in order to assess the effectiveness of the sampling and screening strategies in identifying and recruiting the projected number of women into the study. Screening in this group is complete and data are currently available for analysis. Overall, study participation is on target. The number of women enrolled in the study from the "First Fifty Study" was expected to be 57, while the actual number is 62. Of those screened, 36% were expected to be disabled in two or more domains of function; in fact, the percentage was 37.6%. The age distribution of study participants is close to that projected, with 38% in each of the 65 to 74 and 75 to 84 year groups, and 25% aged 85 and over. A slightly higher than expected percentage of the sample, which is identified through HCFA records, has been found to be deceased or institutionalized. Refusals are higher than expected at the screener stage, but lower than expected among those eligible for the study after screening. Women in the oldest age group (85 and over) were more likely than younger women to refuse screening, but of those in this age group who were screened, two-thirds were disabled in two or more domains and eligible for the study. The data indicate that the overall yield of the screening process is on target, even though projected estimates for some parameters were higher or lower than what is being encountered in the field. Based on these results, no changes will be made in the screening strategy. The analyses will be repeated when the first replicate is completed to verify the findings.

EDBP staff, working in collaboration with Johns Hopkins investigators and the Westat subcontractor, have played an important role in the developmental and field work for this study, including recent work on the development of the follow-up assessment and the proxy follow-up interviews, to be used when participants are unable to report for themselves and when participants have died. Nurses and interviewers have been trained by EDBP staff to conduct the performance measures of functioning as well as ongoing quality control in this area. EDBP staff facilitated working with HCFA to obtain the Medicare beneficiary files for the study sampling and the Part A and B utilization data for analysis. Staff also coordinated study efforts to obtain readings of the participant spirometry measurements from NIOSH. A Manual of Operations is being prepared to provide an overview of the study and the sources and references for all the data collection instruments and procedures as well as the documentation for all the analytic variables in the study. EDBP staff have also directed the creation of the drug data base for the study.



## CONTRACT

Name and Number: University of Iowa (N01-AG-0-2106)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: \$328,945

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged.

Methods Employed: The project includes cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population is included.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. To provide new knowledge, it is important to study representative community-dwelling populations. Within obvious logistical constraints populations will be available to the NIA scientific community for specific studies.

Proposed Course: Continued surveillance during a 5-year period (1989-93) will be based on the use of the National Death Index for mortality and the Health Care Financing Administration's Medicare data for morbidity. While contract closeout will occur this calendar year, intensive research efforts will continue.

Major Findings: Prospective evidence for predictors of decline in social relationships over a 3-year follow-up period in an elderly cohort are presented. The cohort consisted of men (n=903) and women (n=1,673) over age 65 years in two rural Iowa counties who were interviewed in 1982 and again in 1985. Three separate measures of social relationships were dichotomized into lower and higher levels and included the number of close friends and relatives (less than three vs. three or more), church attendance (less than once per month or not at all vs. once per month or more), and membership in a group (nonmember vs. member). Those with higher social relationship levels at both interviews were compared with those who had higher levels at baseline but lower levels at follow-up (i.e., a decline in social relationship level) using logistic regression. In multivariate analysis, important baseline predictors of decline in social relationship levels included greater age, lower education level, lower memory test score, the presence of physical disabilities, and a higher level of depressive symptoms. Marital status and lower self-perceived health status were less consistent predictors, and having any living children, history of major illness, and continence



status were generally not important predictors of decline in social relationship levels. These findings underscore the multifactorial and complex influences on changes in social relationships, but they also identify factors for possible prevention and intervention strategies (ref. 1).

To describe the incidence and health resource consequences of self-reported adverse drug reactions (ADRs) and their relation to self-perceived health, number of medications used, and age in a geographically based population 65 years of age and older. A survey of a defined population. Three thousand, one hundred seventy noninstitutionalized persons 65 years of age and older residing in two Iowa counties. Reports of ADRs (elicited using an open-ended questionnaire); names of drugs involved; descriptions of reactions and related health resource use; self-perceived health status (at the first annual follow-up); and number of medications (from the baseline interview). Ten percent (95% CI, 8.97 to 11.09) of respondents reported one or more ADRs. Three quarters of respondents who reported ADRs contacted a physician. Of these, half indicated that a laboratory test had been ordered, and 7% reported a hospitalization due to the reaction. Advanced age alone was associated with decreased risk in women; a similar trend in men was not statistically significant. However, persons with poorer health status and those who reported the greatest prior use of medications were most likely to report reactions. In this study of noninstitutionalized elderly persons, advanced age did not appear to be associated with increased risk for self-reported ADRs. We could not determine whether the decrease in ADR reports among the oldest respondents represented true diminished ADR occurrence or altered ADR detection and reporting capabilities. When projected to the elderly community-dwelling U.S. population, 2.2 million annual physician visits, 1.1 million laboratory tests, and 146,000 hospitalizations may result from ADRs (ref. 2).

Depressive symptomatology was examined in a large sample of noninstitutionalized older adults using the Center for Epidemiological Studies-Depression scale (CES-D). Both cross-sectional and longitudinal data showed age-related increases in mean CES-D scores and increases in the percent age of respondents scoring at or above the cutoff score of 16. Variables collected at baseline in the longitudinal study from 2,032 participants 65 years of age and older were significant predictors of depressive symptomatology 3 and 6 years later. Baseline CES-D scores accounted for the largest proportion of the variance (ref. 3).

Driving requires the continuous integration of sensory, cognitive, and motor skills, many for which are subject to age and illness-associated impairments. This article reviews functions related to driving, the methods used for their measurement, the epidemiology of age-associated functional impairments, and the relationship of functional impairments to driving behavior and legal interventions. Analyses presented here indicate that although physical functional impairments appear to be associated with both voluntary changes in driving habits and legally imposed regulations, cognitive impairments are associated with legally imposed restrictions but only modest voluntary changes. Suggestions are made for further research aimed at developing reliable and valid screens for functionally impaired drivers and interventions that will maintain or enhance the skills of already impaired drivers (ref. 4).



## Publications

1. Cerhan JR, Wallace RB. Predictors of decline in social relationships in the rural elderly. Am J Epidemiol 1993;137:870-80.
2. Chrischilles EA, Segar ET, Wallace RB. Self-reported adverse drug reactions and related resource use: A study of community-dwelling persons 65 years of age and older. Ann Intern Med 1992;117:634-40.
3. Wallace J, O'Hara MW. Increases in depressive symptomatology in the rural elderly: Results from a cross-sectional and longitudinal study. J Abnormal Psychology 1992;101:398-404.
4. Colsher PF, Wallace RB. Geriatric assessment and driver functioning. Clinics in Geriatric Medicine 1993;9(2):356-73.





## CONTRACT

Name and Number: Yale University (NO1-AG-O-2105)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: \$359,749

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged.

Methods Employed: The project includes cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population is included.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. To provide new knowledge, it is important to study representative community-dwelling populations. Within obvious logistical constraints populations will be available to the NIA scientific community for specific studies.

Proposed Course: Continued surveillance during a 5-year period (1989-93) will be based on the use of the National Death Index and the Health Care Financing Administration's Medicare data for morbidity. While contract closeout will occur this calendar year, intensive research efforts will continue.

Major Findings: Of the 120 cohort members who sustained a hip fracture in the 6-year study period, 22 died within 6 months of the fracture. Among survivors there was a sustained decline in function at 6 weeks after the fracture with little improvement by 6 months. At baseline, 86% could dress independently versus 49% at 6 months; 90% could transfer independently versus 32% at 6 months; 75% could walk across a room independently versus 15% at 6 months; 63% could climb a flight of stairs versus 8% at 6 months; and 41% could walk one-half mile versus 6% at 6 months. Physical function and mental status were the only baseline factors significantly associated with physical function at 6 months after the fracture in bivariate analysis, while physical function and depression were associated in multivariate analysis (ref. 1).

The influence of selected psychosocial factors as predictors of stroke incidence were studied in this cohort. The main psychosocial factor of interest was depression. Marital status, social support, social networks, and religiousness were also assessed as potential antecedent



or mediating factors. The data were based on 2,812 individuals aged 65 years and over living in New Haven, Connecticut. The incidence of stroke was monitored from the baseline interview in 1982 until December 1988. Depression, measured by the Center for Epidemiologic Studies Depression Scale (CES-D), was measured at baseline as were other predictor variables. Univariate Cox regression analyses revealed that higher CES-D scores were predictive of greater stroke incidence ( $p < 0.05$ ). More frequent attendance at religious services was associated with lower incidence ( $p < 0.001$ ). CES-D scores were also correlated with many measures of sociodemographic, health, and physical function factors in our multivariate analysis ( $p < 0.05$ ). When combined with other significant predictor variables such as age, sex, hypertension, diabetes, diabetes, physical function, and smoking, neither depression nor religious attendance retained its significance (ref. 2).

The survival of elderly patients hospitalized for acute myocardial infarction who have emotional support was compared with that of patients who lack such support, while controlling for severity of disease, comorbidity, and functional status. Of 194 patients, 76 (39%) died in the first 6 months after myocardial infarction. In multiple logistic regression analyses, lack of emotional support was significantly associated with 6-month mortality (odds ratio, 2.9; 95% CI, 1.2 to 6.9) after controlling for severity of myocardial infarction, comorbidity, risk factors such as smoking and hypertension, and sociodemographic factors. When emotional support was assessed before myocardial infarction, it was independently related to risk for death in the subsequent 6 months (ref. 3).

Physical function was assessed as a predictor of stroke incidence for the noninstitutionalized elderly subjects with no previous history of stroke. Incidence of stroke was monitored from the baseline interview in 1982 until December 1988 ( $n=167$ ). Physical function was measured by the Katz scale of activities of daily living and a three-item scale measuring gross mobility function (Rosow scale). Both measures of impairment of function were independently associated with stroke incidence controlling for age, sex, diabetes, hypertension, and angina ( $p < 0.001$ ). Our findings suggest that in elderly persons, physical disability is a newly identified risk factor for stroke (ref. 4).

Mortality risk during early bereavement was examined in the New Haven sample of 1046 married elderly persons 65 years and over, followed from 1982 to 1988. Cox' regression models with time-dependent covariates were computed to estimate mortality risk, while controlling for pre-widowhood sociodemographic and health-related variables. Elderly young-old (65-74) and old-old men ( $\geq 75$ ) showed slightly elevated age-adjusted relative risks (RR) during the first 6 months of widowhood (RR=1.69; 95% CI:0.86-3.31 and RR=1.79; 95% CI:0.44-7.28 respectively). These RRs increased slightly after adjustment for pre-widowhood control variables. The age-adjusted RR during early widowhood for young-old women was 2.87 (95% CI: 0.81-2.42), which increased to 3.86 (95% CI:1.11-13.45) after adjustment for sociodemographic and health-related variables. This analysis stresses the usefulness of Cox' regression models with time-dependent covariates to calculate mortality risk for variable periods after onset of widowhood adjusted for pre-widowhood



characteristics. However, the power of the study was limited, resulting in mostly insignificant risk estimates and wide confidence intervals. (ref. 5).

#### Publications

1. Marottoli RA, Berkman LF, Cooney LMJr. Decline in physical function following hip fracture. *J Am Geriatr Soc* 1992;40:861-866.
2. Colantino A, Kasl SV, Ostfeld AM. Depressive symptoms and other psychosocial factors as predictors of stroke in the elderly. *Am J Epidemiol* 1992;136:884-894.
3. Berkman LF, Leo-Summers L, Horwitz RI. Emotional and survival after myocardial infarction: A prospective, population-based study of the elderly. *Ann Intern Med* 1992;117:1003-1009.
4. Colantino A, Kasl SV, Ostfeld AM. Level of function predicts first stroke in the elderly. *Stroke* 1992;23:1355-1357.
5. Mendes de Leon CF, Kasl SV, Jacobs S. Widowhood and mortality risk in a community sample of the elderly: A prospective study. *J Clin Epidemiol* 1993;46:519-527.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Honolulu Aging Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Lon R. White, M.D., M.P.H., Chief, Asia-Pacific Office, EDBP

Richard J. Havlik, M.D., M.P.H., Associate Director, EDBP

Robert Garrison, M.D., Chief, Field Studies Branch, EBP, NHLBI

G. Webster Ross, M.D., Project Neurologist, DVA

Carolyn Murdaugh, Ph.D., Senior Investigator, NCNR

## COOPERATING UNITS (if any)

National Heart Lung and Blood Institute (NHLBI)

National Center for Nursing Research (NCNR)

Department of Veterans Affairs (DVA)

## LAB/BRANCH

Asia-Pacific Office

## SECTION

Epidemiology, Demography, and Biometry Program

## INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

## TOTAL MAN-YEARS:

.9

## PROFESSIONAL:

.85

## OTHER:

.05

## CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) The NIA supplements a research project sponsored by the NHLBI and supported through an NHLBI contract with Kuakini Medical Center in Honolulu, Hawaii, to allow for research on aging and dementia among study participants. The Honolulu Heart Program (HHP) is a prospective study of cardiovascular diseases of American men of Japanese ancestry born from 1900 to 1919 and living on the island of Oahu in 1965. This study will focus on aging, with the emphasis on Alzheimer's disease and multi-infarct dementia. About 3,800 of the approximately 4,600 HHP participants have been contacted thus far, including approximately 3,500 who have received all or almost all of the standard examination and interview. Approximately 20 to 25 minutes of the NHLBI examination is devoted to NIA data collection, including administration of the Cognitive Abilities and Screening Instrument (CASI) and a questionnaire to elicit information concerning possible risk factors for dementia. Approximately 13% of the examined participants were invited back for a follow-up examination. Those invited back include all of the participants who receive low CASI scores, about 33% of those who received borderline scores (74-82, N=176), and 7% of those who received normal (>82, N=165) CASI scores. The purpose of this call-back examination is to carry out a highly standardized dementia evaluation following guidelines previously agreed upon and from which an algorithmic dementia classification can be accomplished with a minimum of subjective interpretation and clinical judgement.





PROJECT NUMBER Z01 AG 07060 02 EDBP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Mediators of the Disease-Weight Loss Relation Among Older Persons in the Framingham Heart Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Tamara Harris, M.D., M.S. Chief, Geriatric Epidemiology Office, EDBP, NIA

Ronald Prior, Ph.D., Scientific Program Officer, Human Nutrition Research Center on Aging

Irwin Rosenberg, M.D., Director, Human Nutrition Research Center on Aging at Tufts University

COOPERATING UNITS (if any)

USDA-Agricultural Research Service

LAB/BRANCH

Geriatric Epidemiology Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

.05

.05

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

X (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this agreement is to fund the drawing, transporting, processing and analysis of blood specimens from the Framingham Heart Study cohort in Examination Cycle 22 and the collection of bioelectric impedance data from the members of the Framingham Heart Study cohort in Examination Cycle 22.

Data collection in Framingham is moving along well. The measurement of cytokine levels in mononuclear cells at Tufts, levels of inflammatory proteins and associated other biochemistries has been successful. Methodologic work over the year suggests that the delay of an hour in laboratory processing while the specimens are being transported has some effect on the measurements but that data from healthy younger persons in Framingham should allow some adjustment. Preliminary data on both stimulated and unstimulated levels of IL-1, TNF and IL-6 shows fairly normal distributions of secretors and non-secretors for each cytokine. Preliminary analyses are ongoing and data collection should be complete this fall.



## CONTRACT

Name and Number: Peter Bent Brigham Hospital (NO1-AG-0-2107)

Title: Established Populations for the Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: \$347,419

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged.

Methods Employed: The project includes cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population is included.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. To provide new knowledge, it is important to study representative community-dwelling populations. Within obvious logistical constraints populations will be available to the NIA scientific community for specific studies.

Proposed Course: Continued surveillance during a 5-year period (1983-93) will be based on the use of the National Death Index for mortality and the Health Care Financing Administration's Medicare data for morbidity. While contract closeout will occur this calendar year, intensive research efforts will continue.

Major Findings: Mortality associated with handedness was examined in two ways. A simulation using national data suggests that lower mean age at death among left-handed persons, previously offered as evidence of higher mortality, can be explained exclusively by the age distribution of laterality. Second, empiric evidence from the East Boston EPESE study of 3,774 older adults demonstrates that left-handedness is not associated with mortality (relative odds=1.04% confidence interval=0.79%, 1.39) (ref. 1).

### Publications

1. Salive ME, Guralnik JM, Glynn RJ. Left-handedness and mortality. *Am J Public Health* 1993;83:265-67.



## CONTRACT

Name and Number: Duke University Medical Center (N01-AG-1-2102)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: January 1, 1991

Current Annual Level: \$959,790

Objectives: The purpose of this project is to conduct epidemiologic investigations in a biracial elderly population, 65 years of age and older, selected from both urban and rural locations.

Methods Employed: Descriptive and analytical epidemiologic studies of existing problems and surveillance of newly developing problems all with an emphasis upon future intervention and prevention have been conducted. Investigators conducted cross-sectional and prospective studies as well as more detailed problem-related studies in a carefully defined and accessible population using standard field and analytical techniques.

Significance to Biomedical Research: The NIA began funding three population studies of the elderly to determine the influences of social, environmental, behavioral, and economic forces on the mortality, morbidity, and utilization of health services in the elderly. These studies, however, were not fully representative of the U.S. elderly; specifically, they did not include a significant proportion of blacks. It is well known that distributions of certain risk factors and diseases differ between U.S. blacks and other racial groups. Therefore, the purpose of the 1984 contract was to conduct epidemiologic investigations in an elderly population of which at least 50 percent is black in order to develop new knowledge concerning the medical and social factors in health and diseases of the aging black population. In addition, both black and white subgroups in the study exhibit an excellent distribution on indicators of socioeconomic status, and participants have been selected from both urban and rural locations.

Proposed Course: A new 7-year contract was awarded to Duke University Medical Center January 1, 1991, to conduct a third in-person survey wave and continue surveillance for major endpoints. In this wave, begun in May 1992, Duke is gathering information comparable to that obtained by the other EPESE sites as well as items which are important for the study of the health of older black persons and racial and urban/rural differences. Physical performance measures being taken include tests of balance, a timed walk, chair stands, a test of shoulder range of motion, and functional reach. Waist/hip ratio, height and weight, and vision are also being ascertained. Blood assays include complete blood count, automated serum chemistries, and HDL cholesterol.



The interviewing has just been completed for the third in-person survey, but collection of blood samples from the participants is still under way, lagging the interviews by several weeks. It is expected that the last samples will be drawn early in the fall. Interview completion rates have exceeded 95 percent throughout this wave, and response to the blood draw has held between 75 percent and 80 percent. In addition to the standard protocols for analysis of blood samples done at the first three EPESE sites, the Duke site is including three measures of immune function, Interleukin-6 (IL-6), cross-linked fibrin degradation products (XDP fibrin D-dimers) and serum protein electrophoresis (SPE) measures of immune globulins. At the present time for the whole cohort 1,530 plasma samples have been collected, 1,045 have been analyzed for XDP, 950 for IL-6 and 700 for SPE.

Once the fieldwork for in-person III is completed, the Duke site will begin concentrated efforts on matching the cohort to the Medicare hospitalization files of HCFA and the National Death Index, as has been done in the other three EPESE sites. In addition, Duke will maintain surveillance of mortality and nursing home admissions through contacts with the cohort and other means used in the other sites. This work will continue for a period of 4 years.

**Major Findings:** Hypertension in blacks, compared with whites,<sup>1</sup> occurs at higher prevalence rates, is more severe, and carries a worse prognosis for cardiovascular morbidity and mortality. The authors examined the degree to which black/white differences in hypertension in the elderly are explained by demographic variables, income, health behavior (smoking, obesity), health service use, and comorbid diabetes. The study population consisted of subjects participating in the Duke EPESE, initiated in 1984. Cross-sectional data reported here were collected between January 1986 and July 1987. Subjects were aged 65 years or older and were not institutionalized. Blacks were oversampled. Of 5,223 eligible persons, 4,163 (80%) agreed to be interviewed; 16% of the study subjects were white men, 30% white women, 19% black man, and 35% black women. The mean age for all groups was approximately 73 years. Forty-four percent of white men, 52% of the white women, 50% of black men, and 66% of black women had hypertension. Eighty percent of hypertensives were receiving pharmacologic therapy. Older age, female sex, lower socioeconomic status, obesity, and diabetes mellitus were associated with hypertension. After adjusting for covariables, black race/ethnicity remained an independent risk factor for high blood pressure in the elderly, with an adjusted odds ratio of 1.30 (ref.1).

Epidemiologic surveys, experimental studies in animals, and clinical trials in young and middle-aged patients with hypertension indicate that dietary potassium lowers blood pressure. The mechanism of the antihypertensive effect is not well defined. Variations in serum potassium within the physiologic range may directly affect vascular smooth muscle tone. Potassium may also influence the regulation of blood pressure through effects on sodium handling, aldosterone secretion, the renin/angiotensin system, renal kallikrein, eicosanoids, and atrial natriuretic peptide. This study was undertaken to confirm the blood pressure-lowering effect of potassium in older patients and to determine the mechanism of the antihypertensive effect. Twenty-two patients  $\geq 60$  yr of age were admitted to a Clinical





Research Unit for 8 days after a 2-wk period free of antihypertensive medication. Patients were placed on an isocaloric diet containing 200 mmol/day of Na<sup>+</sup>, 70 mmol/day of K<sup>+</sup>, and 500 mg/day of Ca<sup>2+</sup> and were treated in a randomized, double-blinded manner with either potassium chloride (120 mmol/day) or placebo. After 4 days, patients were crossed over to the alternate treatment. Systolic blood pressure decreased 8.6 mm Hg (95% confidence interval -14.6, -2.6), and diastolic blood pressure decreased 4.0 mm Hg (-6.9, -1.0) during potassium chloride supplementation. There was no significant change in blood pressure during treatment with placebo. Serum K<sup>+</sup> was 3.9 ± 0.1 mmol/L after 3 days of placebo and 4.3 ± 0.1 after 4 days of potassium chloride (P < 0.0002). Urinary sodium excretion averaged 192 ± 11 mmol/day after placebo and 221 ± 8 after potassium treatment (P < 0.002). Potassium treatment was not associated with changes in supine or captopril-stimulated PRA, GFR, atrial natriuretic peptide level, or urinary excretion of thromboxane B<sub>2</sub> or 6-keto-prostaglandin F<sub>1m</sub>. It was concluded that potassium chloride lower blood pressure and increased sodium excretion in older patients with mild hypertension. The blood pressure effect may be due in part to potassium-induced natriuresis (ref. 2).

Information on prescription and over-the-counter drug use, demographic and health characteristics, and use of health services was obtained from a probability-based sample of black (n=2,152) and white (n=1,821) community-resident elderly in the Piedmont area of North Carolina. Descriptive statistics were calculated. Linear regression, in which sample weights and design effects were taken into account, was used for the final models. For prescription drug use, 37% and 32% of the variance was explained for whites, and blacks, respectively (6% and 5% for over-the-counter drugs). Health status and use of medical services were the strongest predictors of prescription drug use for both races (with Medigap insurance also important for white and Medicaid for blacks). Demographic characteristics and self-assessed health were significant factors in use of over-the-counter drugs. Race independently predicted use of both types of drugs, but explained only a small proportion of the variance. The study concluded that health status and use of health services are important in explaining prescription drug use. Over-the-counter drug use is difficult to explain (ref. 3).

The prevalence of people and sites with attachment loss, pocket depth, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, and *Porphyromonas gingivalis* are described for a random sample of 366 black and 297 white community-dwelling adults, aged 65 or over, residing in 5 counties in North Carolina. In addition, relationships between sites harboring these microorganisms and loss of attachment (LA) and pocket depth (PD) are presented in a manner that considers the lack of independence of sites within each person. Pocket depths and recession were measured on all teeth by trained examiners during household visits. Immunofluorescent assays for *A. actinomycetemcomitans*, *P. intermedia*, and *P. gingivalis* were conducted on subgingival plaque samples obtained from the mesiobuccal aspect of the four first molar teeth using paper points. The prevalences of *A. actinomycetemcomitans*, *P. intermedia*, and *P. gingivalis* were greater in blacks than in whites. The most striking difference was seen for *P. gingivalis*, which was found in 38.8% of blacks and 9.4% of whites. Similar relationships were found when the percent of sites with these organisms were assessed. Blacks with *P. gingivalis* or *P. intermedia* had a higher



prevalence of sites with  $LA \geq 7$  mm as compared to blacks not infected with *P. gingivalis* or *P. intermedia*. The same was true for whites. Similar relationships between *P. gingivalis* or *P. intermedia* and  $PD \geq 6$  mm were found for both blacks and whites. However, the greater prevalence for blacks than whites for  $LA \geq 7$  mm or  $PD \geq 6$  mm when both were infected with *P. gingivalis* or *P. intermedia* were suggestive trends rather than significant. Finally, a logistic regression model for  $LA \geq 7$  mm showed that race was no longer a significant explanatory variable when *P. gingivalis*, *P. intermedia*, last visit to the dentist being more than 3 years ago, and tobacco use were already in the model. It is clear that destructive periodontal disease is the result of a complex relationship among subgingival microflora and non-bacterial factors. The interaction between the microbial flora and these other factors present a fruitful area of investigations in order to fully understand the complexity of periodontal disease in adults (ref. 4).

### Publications

1. Svetkey LP, George LK, Burchett BM, Morgan PA, Blazer DG. Black/white differences in hypertension in the elderly: An epidemiologic analysis in central North Carolina. Am J Epidemiol 1993;137:64-73.
2. Smith SR, Klotman PE, Svetkey LP. Potassium chloride lowers blood pressure and causes natriuresis in older patients with hypertension. J Am Soc Nephrol 1992;2:1302-1309.
3. Fillenbaum GG, Hanlon JT, Corder EH, Zigebu-Page T, Wall WH, Brock D. Concomitants of prescription and over-the-counter drug use in black and white community resident elderly. Am J Public Health (in press).
4. Beck JD, Koch GG, Zambon JJ, Genco P<sup>T</sup>, Tudor GE. Evaluation of oral bacteria as risk indicators for periodontitis in older adults. Periodontol 1992;63:93-99.



PROJECT NUMBER Z01 AG 07030 05 EDBP  
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

**PERIOD COVERED**

October 1, 1992 to September 30, 1993

**TITLE OF PROJECT** (80 characters or less. Title must fit on one line between the borders.)

NHANES III: Health of Older Person (Baseline Survey)

**PRINCIPLE INVESTIGATOR** (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Richard J. Havlik, M.D., M.P.H., Associate Director, EDBP NIA

Kathy Lonsonczy, M.S., Biometry Office, EDBP, NIA

Robert Murphy, Division of Health Examination Statistics, NCHS

**COOPERATING UNITS** (if any)

National Center for Health Statistics

**LAB/BRANCH**

Biometry Office

**SECTION**

Epidemiology, Demography, and Biometry Program

**INSTITUTE AND LOCATION**

NIA, NIH, Bethesda, MD 20892

**TOTAL MAN-YEARS:**

.05

**PROFESSIONAL:**

.05

**OTHER:**

**CHECK APPROPRIATE BOX(ES)**

☒ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

**SUMMARY OF WORK** (Use standard unreduced type. Do not exceed the space provided.)

NHANES III is a multi-agency collaborative survey designed to estimate the prevalence of diseases and risk factors in some 30,000 Americans and conducted by the National Center for Health Statistics. Special efforts are being directed to collection of data from interviews and examinations for the population over age 60 and the oldest-old.

Phase I of NHANES III, a national sample in itself and about one-half of the projected total, is completed. The overall examination rate is 78.7 percent and this rate is higher than for NHANES II. However, the response rates are somewhat lower for those 75 years and older. Home examinations are being done to improve the participation rate in older persons.

Biomedical examination content of particular interest to EDB Program investigators includes 24-hour dietary recalls, bioelectrical impedance for body composition, bone density measurement, knee x-rays, and an assessment of physical functioning. Phase I data files are becoming available for preliminary use. Phase II of NHANES III data collection is continuing at the same time and early participation rates have been high.



PROJECT NUMBER Z01 AG 07050 02 EDBP  
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

1993 National Mortality Followback Survey

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Dwight B. Brock, Ph.D., Chief, Biometry Office, EDBP, NIA

Eleanor Simonsick, Ph.D., Epidemiology and Demography Office, EDBP, NIA

Paul Placek, Ph.D., Chief, Followback Survey, NCHS

COOPERATING UNITS (if any)

National Center for Health Statistics

LAB/BRANCH

Biometry Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

.08

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☐ (a1) Minors

☒ (a2) Interviews

☐ (b) Human tissues

☐ (c) Neither

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this agreement is to support the collection and analysis of data on cause of death and characteristics of the last year of life in the planning of the 1992 Pretest and 1993 Main Survey of the 1993 National Mortality Followback Survey (NMFS), conducted by NCHS, CDC. This survey will supplement information from death certificates in the vital statistics file with information on characteristics of the decedent. The pretest will examine approximately 800 deaths of individuals aged 15 years and over who died in 1992. The main survey will examine approximately 20,000 deaths of individuals aged 15 years and over who died in 1993. This will include 1,000 deaths to centenarians.

A pretest has been conducted in four states, establishing the feasibility of the types of questions to be investigated in the main survey and showing that high levels of response can be expected from the informants. Upon completion of the analysis of the pretest data, final versions of the questionnaire will be prepared and the sampling of death certificates for the main study will begin. It is expected that the majority of the interviews will be conducted by telephone, as that mode was used successfully in the pretest and appeared to provide high quality data at substantially lower costs than face-to-face interviews. The interviewing will be done by Census Bureau interviewers, beginning in January of 1994. It is expected that data collection will continue for approximately one year, and that a final data tape will be available for analysis about one year after the data collection is completed.

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